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## ORIGINAL ARTICLE

# Stability of Thirty-Four Analytes in Blood Samples of Diabetic Patients

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**SUMMARY**

***Background:*** **Storage of biological samples may alter the values of an analyte compared to that of initial measurement. Therefore, an optimal storage condition for every analyte in serum and whole blood samples needs to be determined. The aim of this study was to investigate stability of 34 analytes at different time and temperature conditions of storage.**

***Methods:*** **This study assessed the stability of hematological parameters in whole blood sample and common biochemical analytes in serum of 40 diabetic patients after 24 and 48 hours in 2 - 8°C and after 30 days in -20°C of sample collection. The mean values of analytes in 3 different storage conditions were measured and compared to that of initial values.**

***Results:*** **Most of the examined biochemical analytes and hematological parameters were stable up to 48 hours at 2 - 8°C after sample collection. Most of the negative changes were negligible but PTH level dramatically decreased after 48 hours in 2 - 8°C. In addition, although a clear increase in the concentration of triglycerides, Cr, Urea, T4, and 25-OH vitamin D3 was observed, it was not significant. Furthermore, a statistically significant difference was observed in the values of ALT, Ca, and T4 among the different conditions of storage. Also, values of HbA1c did not show any significant statistical changes among the 3 different conditions of storage.**

***Conclusions:*** **Taken together, it seems that most of the analytes in the serum of diabetic patients as well as HbA1c are stable up to 30 days of storage.**

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**KEY WORDS**

diabetes, stability, biochemical analytes, hematological parameters, HbA1c

## INTRODUCTION

Diabetes mellitus is one of the major global health problems and it is estimated that the number of patients between 2013 and 2045 will increase from 382 million to 629 million patients. The maximum rise in patient numbers and burden is expected in developing countries [1]. Prevalence of diabetes in Iran is 9.3% in adult population (adjusted to national population) and the total number of diabetic patients will be nearly doubled (4,695 to 8,384 in 1,000s) within the next 12 years [2].

Numerous epidemiologic, clinical and basic research has been conducted globally about different aspects of diabetes [3-5], most of them are based on laboratory results. Some laboratory parameters such as HbA1c are too important for disease control like diabetes and for monitoring of disease progress and outcome as well [6]. Measurement of these laboratory parameters by different assays may not result in reliable results as it has been shown that results from some HbA1c assays are not acceptable for classification of diabetic patients by HbA1c level [7]. In addition, the difference in performance of the devices used for measurement of these parameters may cause imprecision of results as it was found for HbA1c that there are still unacceptable errors in HbA1c and further improvements are required to standardize HbA1c measurements [8].

One of these effective factors in the reliability of results is sample storage condition. The quality of biological samples is crucial for the reliability of laboratory results for research and management of diseases like diabetes. Routinely in clinical laboratories, blood samples are processed after specimen collection and stored at a suitable temperature until analysis and even after analysis in order to do additional tests according to the patient's condition [9]. In any type of research, especially epidemiologic studies, it is difficult to examine samples immediately after phlebotomy and often samples need to be transported to the central laboratory for examination and biobanking. For example, in UK biobank, which is a large national project in the UK, samples are delivered within 30 hours [10-12].

Any laboratory should consider the best stable storage condition for each analyte in case of probable repetition in measurement. In clinical laboratories, samples are stored at 2 - 8°C in a refrigerator or in a -20°C freezer. The stability of several analytes following processing of samples and serum separation has been described previously; however, information is often inadequate and sometimes inconsistent [9,13]. According to the International Organization for Standardization guide, a sample is considered stable when it does not exceed the limited range from initial measurement by passing the time [14], but it is required to determine the exact stable time for each analyte in different types of samples. Therefore, the quality evaluation of samples in every research in each country with a different protocol of sampling is required. This project was conducted in Iran which is a large country and transfer of samples from the entire country to the laboratory within 30 hours may be difficult. This study aim was to evaluate the stability of various biochemical and hematological parameters during a period of time under a specified storage condition. All the guidelines for blood sample handling and separation of serum were taken into account.

## MATERIALS AND METHODS

### Subjects and collection tubes

In this cross-sectional study, blood specimens were collected from a total of 40 diabetic patients referred to the laboratory of Diabetes and Metabolic Diseases Clinic of Tehran University of Medical Sciences for follow up of diabetes. Becton Dickinson (BD) vacutainer plain plastic tubes with clot activator and plastic whole blood tube coated with K2EDTA were used for blood collection. Filling and mixing of the tubes collected were performed according to the manufacturer's recommendations. Analytes and instruments used for measurement in this study are shown in Table 1.

The study protocol was approved by the ethics committee at Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences. The study was conducted according to the Helsinki Declaration revision 2013 [15].

### Sample preparation and storage

Plain tubes were centrifuged at 2,600 g for 10 minutes within 60 minutes of phlebotomy and serum samples were allocated into 4 vials. The first vial was used immediately for biochemistry and hormonal analysis (T0).

### Refrigerator temperature

Two vials were stored at 2 - 8°C (RF) for 24 and 48 hours and then analyzed (T24 and T48). One vial of each sample was stored in -20°C for 30 days (T30 days).

For whole blood stability of cell blood count (CBC), analytes were measured immediately (T0) and then tubes were stored at 2 - 8°C for 24 and 48 hours and reanalyzed (T24 and T48).

### Freeze and thaw stability

One vial of each serum and whole blood samples which were stored at -20°C for 30 days were thawed and reanalyzed for biochemical and HbA1c tests.

### Data analysis

Blood and serum samples were analyzed instantly and the baseline results were compared with results which were taken at a given time by ANOVA test and Tukey's Post Hoc. The SPSS software version 25.00 for Windows was used for analysis of results.

The instability of analytes at different conditions was also calculated as a deviation percentage of each result from initial result.

We used two approaches by Oddoze [13] to establish

**Table 1. Analytes and instruments used in this study.**

| | General Biochemistry | Immunoassay | HbA1c | Cell blood count |
|---|---|---|---|---|
| **Instrument** | **Cobas C-311 analyzer (Roche Diagnostic, Germany)** | **Cobas e-411 immunoassay analyzer (Roche Diagnostic, Germany)** | **Tosoh G8 HPLC Analyzer (Tosoh Bioscience, Japan)** | **Sysmex K-21N automated hematology analyzer (Sysmex, Japan)** |
| **Analytes** | **Alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (Ca), total cholesterol (Chol), creatinine (Cr), γ-glutamyltransferase (GGT), glucose (Glu), iron (Fe), lactate dehydrogenase (LDH), magnesium (Mg), inorganic phosphorus (Ph), total protein (TP), triglycerides (TG), urea (Ur), uric acid (UA), HDL and LDL cholesterol** | **Triiodothyronine (T3), Thyroxin (T4), Thyroid Stimulating Hormone (TSH), Ferritin (Fer), 25 Hydroxy Vitamin D (25 OH Vit D), Parathyroid Hormone (PTH)** | **HbA1c** | **White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelet (PLT)** |

performance goals: First, the Acceptable Change Limit (ACL) was measured based on the analytical imprecision (CVa), using the formula ACL = 2.77 CVa where CVa was estimated according to the internal quality control of laboratory over past 4 months. In the second approach, both analytical and intra-individual [16] imprecision were considered and total change limit (TCL) was estimated (square root of the sum of the squared analytic and biologic imprecision).

## RESULTS

The values of ALT, Ca, and T4 in different conditions were significantly different (p-value < 0.05). Tukey's Post Hoc showed that storage of sample for 30 days in -20°C makes the difference among the different conditions (Table 2). We did not observe any significant differences in hematological parameters among different conditions of storage (Table 3).

Percentage difference for each parameter in different conditions is expressed as bias % (Tx-T0/T0) x 100, and it is presented in Tables 4 and 5 and also in Figure 1. All of the examined biochemical and hematological analytes were stable up to 48 hours at 2 - 8°C after sample collection. As expected, changes in ALT values in frozen samples were not acceptable using both TCL and ACL. TG differences were more than ACL criterion (8.6% vs. 6.9%). Changes in Cr, Glu, T4, 25 OH VitD in frozen samples were too close to ACL and TCL. A PTH change in T48 was also close to ACL and TCL while the differences were too small for T24 and frozen samples. Also, values of HbA1c did not show any statistical or clinical changes among the 3 different conditions of sample storage. Figure 1 shows percentage change in concentration of blood parameters per 24 hours stored.

## DISCUSSION

We examined the effects of storage condition on the concentration of biochemical and hematological parameters in whole blood and serum samples of diabetic patients. The instability of samples was determined by calculation of ACL and TCL parameters where if the mean of the difference of an analyte exceeds the ACL or TCL, it was determined as unstable for that specific storage condition. In our study, most of the analytes were stable up to 48 hours at 2 - 8°C after sample collection. The examined biochemical parameters were also stable after 30 days at -20°C. This means that in Iranian epidemiological studies it is possible and safe to send samples to the central lab for laboratory analysis and also biobanking within 48 hours of sampling.

In this study, most of the biochemical parameter levels mildly increased in 2 - 8°C which may be due to evaporation, except for AST, LDL, LDH, PTH, and TSH all of which decreased. Most of the negative changes are negligible but PTH dramatically decreased in 2 - 8°C after 48 hours (-11.85%) but still the change was less than ACL and TCL. Similar studies have reported controversial results which are mostly due to acceptance criteria and also type of samples used (serum or EDTA plasma) [13,17,18].

We also observed increasing values for biochemical analytes in frozen samples; however, considering within laboratory variation, the majority of positive biases were much less than ACL and TCL criteria. A clear increase in the concentration of TG, Cr, Urea, T4, and 25

**Table 2. Difference of analytes in different conditions of sample storage.**

| Analytes | Initial measurement range | Initial measurement mean (SD) | 24 hours (RF) mean (SD) | 48 hours (RF) mean (SD) | 30 days (-20°C) mean (SD) | p-value |
|---|---|---|---|---|---|---|
| HbA1c (%) | 4.5 - 13.7 | 7.39 (1.56) | 7.45 (1.59) | 7.45 (1.59) | 7.45 (1.60) | 0.998 |
| ALB (g/dL) | 3.9 - 5.1 | 4.48 (0.28) | 4.52 (0.29) | 4.56 (0.29) | 4.56 (0.31) | 0.541 |
| ALK (U/L) | 43 - 100 | 70.63 (14.32) | 71.28 (14.32) | 72.05 (14.66) | 74.38 (15.17) | 0.682 |
| ALT (U/L) | 8 - 33 | 18.15 (5.83) | 18.15 (5.73) | 17.78 (5.4) | 13.53 (4.50) | 0.000 * |
| AST (U/L) | 10 - 32 | 18.85 (4.57) | 19.18 (4.58) | 19.28 (4.79) | 18.63 (4.72) | 0.920 |
| Ca (mg/dL) | 8.4 - 10.4 | 9.44 (0.42) | 9.56 (0.47) | 9.57 (0.42) | 9.73 (0.50) | 0.046 |
| Chol (mg/dL) | 89 - 251 | 151.00 (32.35) | 153.80 (32.65) | 156.00 (33.00) | 149.33 (33.08) | 0.807 |
| Cr (mg/dL) | 0.54 - 5.52 | 0.92 (0.76) | 0.94 (0.77) | 0.94 (0.77) | 1.02 (0.76) | 0.942 |
| Fe (µg/dL) | 26 - 154 | 79.7 (25.11) | 80.45 (25.13) | 81.83 (26.00) | 82.63 (27.05) | 0.957 |
| Fer (ng/mL) | 15 - 287 | 99.83 (77.80) | 100.80 (79.14) | 99.19 (77.05) | 102.09 (80.50) | 0.999 |
| GGT (U/L) | 8 - 42 | 24.38 (10.19) | 24.53 (10.23) | 24.83 (10.35) | 26.80 (10.95) | 0.705 |
| Glu (mg/dL) | 85 - 379 | 146.48 (55.70) | 147.80 (56.92) | 149.63 (57.39) | 153.23 (59.78) | 0.958 |
| HDL (mg/dL) | 27 - 79 | 44.78 (12.52) | 45.48 (12.19) | 46.25 (12.24) | 45.33 (12.66) | 0.962 |
| LDH (U/L) | 91 - 397 | 171.6 (54.73) | 169.20 (54.95) | 164.80 (53.30) | 175.18 (56.67) | 0.860 |
| LDL (mg/dL) | 44 - 185 | 85.2 (29.31) | 83.68 (28.92) | 83.75 (29.24) | 85.43 (30.07) | 0.989 |
| Mg (mg/dL) | 1.22 - 2.48 | 1.91 (0.24) | 1.94 (0.24) | 1.97 (0.25) | 1.94 (0.27) | 0.826 |
| Ph (mg/dL) | 2.6 - 5.5 | 3.96 (0.59) | 4.04 (0.59) | 4.03 (0.57) | 4.03 (0.59) | 0.911 |
| PTH (pg/mL) | 23.4 - 222.6 | 52.98 (32.46) | 50.25 (31.40) | 46.75 (29.90) | 52.15 (32.98) | 0.822 |
| T3 (ng/dL) | 71 - 162 | 99.32 (17.50) | 101.04 (18.86) | 102.71 (17.70) | 98.21 (16.97) | 0.688 |
| T4 (µg/dL) | 5.7 - 10.3 | 7.66 (1.15) | 7.79 (1.24) | 7.69 (1.15) | 8.81 (1.36) | 0.000 |
| TG (mg/dL) | 43 - 401 | 138.55 (84.98) | 141.83 (84.75) | 145.25 (85.96) | 150.48 (88.31) | 0.936 |
| TP (g/dL) | 6 - 8.2 | 7.1 (0.46) | 7.15 (0.48) | 7.21 (0.48) | 7.35 (0.55) | 0.129 |
| TSH (µIU/mL) | 0.25 - 5.5 | 2.06 (1.39) | 2.05 (1.38) | 2.05 (1.38) | 2.04 (1.37) | 1.000 |
| UA (mg/dL) | 2.6 - 11.5 | 5.49 (1.73) | 5.54 (1.74) | 5.60 (1.76) | 5.66 (1.77) | 0.973 |
| Ur (mg/dL) | 17 - 97 | 34.68 (15.69) | 35.30 (15.75) | 34.83 (15.96) | 37.23 (16.29) | 0.884 |
| 25 OH Vit D (nmol/L) | 6.4 - 42 | 21.18 (9.70) | 20.99 (10.18) | 21.25 (10.01) | 24.81 (10.60) | 0.270 |

*** - A p-value less than 0.05 was considered as significant difference.**

**Table 3. Statistical difference of hematological parameters in different times of sample storage.**

| Analytes | Initial measurement range | Initial measurement mean (SD) | 24 hours 2 - 8°C mean (SD) | 48 hours 2 - 8°C mean (SD) | p-value |
|---|---|---|---|---|---|
| WBC (* $10^3$/µL) | 3.6 - 10.4 | 6.47 (1.66) | 6.50 (1.71) | 6.49 (1.75) | 0.997 |
| RBC (* $10^6$/µL) | 3.8 - 5.96 | 4.79 (0.46) | 4.78 (0.46) | 4.79 (0.47) | 0.986 |
| Hb (g/dL) | 10.6 - 19 | 14.06 (1.49) | 14.13 (1.51) | 14.13 (1.51) | 0.972 |
| HCT (%) | 33.7 - 48.9 | 40.13 (3.20) | 40.12 (3.08) | 40.34 (3.22) | 0.943 |
| MCV (fL) | 62.1 - 92.9 | 84.06 (5.52) | 84.41 (5.56) | 84.55 (5.61) | 0.920 |
| MCH (pg) | 19.5 - 32.8 | 29.42 (2.52) | 29.72 (2.60) | 29.90 (3.15) | 0.731 |
| MCHC (g/dL) | 31.5 - 38.9 | 34.95 (1.37) | 35.16 (1.46) | 35.03 (1.33) | 0.788 |
| PLT (* $10^3$/µL) | 79 - 387 | 230.13 (69.86) | 229.55 (68.44) | 234.70 (71.28) | 0.937 |

**The p-value less than 0.05 was considered as significant difference.**

**Table 4. Stability of analytes in different conditions of sample storage.**

| Analytes | Analytical CV% | TCL | ACL | Mean of difference% T24h (RF) | Mean of difference% T48h (RF) | Mean of difference % 30 days (-20°C) |
|---|---|---|---|---|---|---|
| HbA1c (%) | 1.5 | 4.3 | 4.16 | 0.71 | 0.78 | 0.74 |
| ALB (g/dL) | 1.6 | 4.7 | 4.43 | 0.78 | 1.79 | 1.84 |
| ALK (U/L) | 2.5 | 7.6 | 6.93 | 0.92 | 2.02 | 5.31 |
| ALT (U/L) | 2 | 11.2 | 5.54 | 0.00 | -2.07 | -25.48 |
| AST (U/L) | 2 | 8.3 | 5.54 | 1.72 | 2.25 | -1.19 |
| Ca (mg/dL) | 1.3 | 3.8 | 3.60 | 1.27 | 1.40 | 3.05 |
| Chol (mg/dL) | 1.2 | 4.5 | 3.32 | 1.84 | 3.29 | -1.13 |
| Cr (mg/dL) | 3.9 | 11.2 | 10.80 | 1.68 | 2.25 | 10.60 |
| Fe (µg/dL) | 2.5 | 14.8 | 6.93 | 0.94 | 2.67 | 3.67 |
| Fer (ng/mL) | 7 | 20.6 | 19.39 | 0.97 | -0.64 | 2.27 |
| GGT (U/L) | 4.7 | 14.6 | 13.02 | 0.62 | 1.85 | 9.95 |
| Glu (mg/dL) | 1.7 | 5.5 | 4.71 | 0.90 | 2.15 | 4.61 |
| HDL (mg/dL) | 1.9 | 6.4 | 5.26 | 1.56 | 3.29 | 1.23 |
| LDH (U/L) | 2.5 | 8.2 | 6.93 | -1.40 | -3.96 | 2.08 |
| LDL (mg/dL) | 2 | 6.8 | 5.54 | -1.79 | -1.70 | 0.26 |
| Mg (mg/dL) | 2 | 5.8 | 5.54 | 1.42 | 2.78 | 1.48 |
| Ph (mg/dL) | 2 | 6.9 | 5.54 | 2.09 | 1.96 | 1.83 |
| PTH (pg/mL) | 5 | 19.0 | 13.85 | -5.14 | -11.75 | -1.57 |
| T3 (ng/dL) | 4 | 11.6 | 11.08 | 1.73 | 3.41 | -1.12 |
| T4 (µg/dL) | 7.8 | 21.7 | 21.61 | 1.62 | 0.29 | 14.94 |
| TG (mg/dL) | 2.5 | 12.1 | 6.93 | 2.36 | 4.84 | 8.61 |
| TP (g/dL) | 2.5 | 7.1 | 6.93 | 0.74 | 1.59 | 3.52 |
| TSH (µIU/mL) | 4 | 14.7 | 11.08 | -0.57 | -0.69 | -1.15 |
| UA (mg/dL) | 1.5 | 6.0 | 4.16 | 1.05 | 2.10 | 3.24 |
| Ur (mg/dL) | 2.7 | 9.6 | 7.48 | 1.80 | 0.43 | 7.35 |
| 25 OH Vit D (nmol/L) | 7 | 19.6 | 19.39 | -0.92 | 0.33 | 17.13 |

**Table 5. Stability of hematological parameters in different conditions of sample storage.**

| Analytes | Analytical CV% | TCL | ACL | Mean of difference % T24h | Mean of difference % T48h |
|---|---|---|---|---|---|
| WBC (* $10^3$/µL) | 0.94 | 6.3 | 2.60 | 0.46 | 0.27 |
| RBC (* $10^6$/µL) | 1.23 | 3.8 | 3.41 | -0.34 | -0.06 |
| Hb (g/dL) | 1.4 | 4.1 | 3.88 | 0.50 | 0.50 |
| HCT (%) | 1.2 | 3.6 | 3.32 | -0.02 | 0.52 |
| MCV (fL) | 0.7 | 2.1 | 1.94 | 0.42 | 0.59 |
| MCH (pg) | 0.7 | 2.1 | 1.94 | 1.03 | 1.66 |
| MCHC (g/dL) | 0.6 | 1.7 | 1.66 | 0.61 | 0.23 |
| PLT (* $10^3$/µL) | 4 | 12.0 | 11.08 | 0.61 | 0.23 |

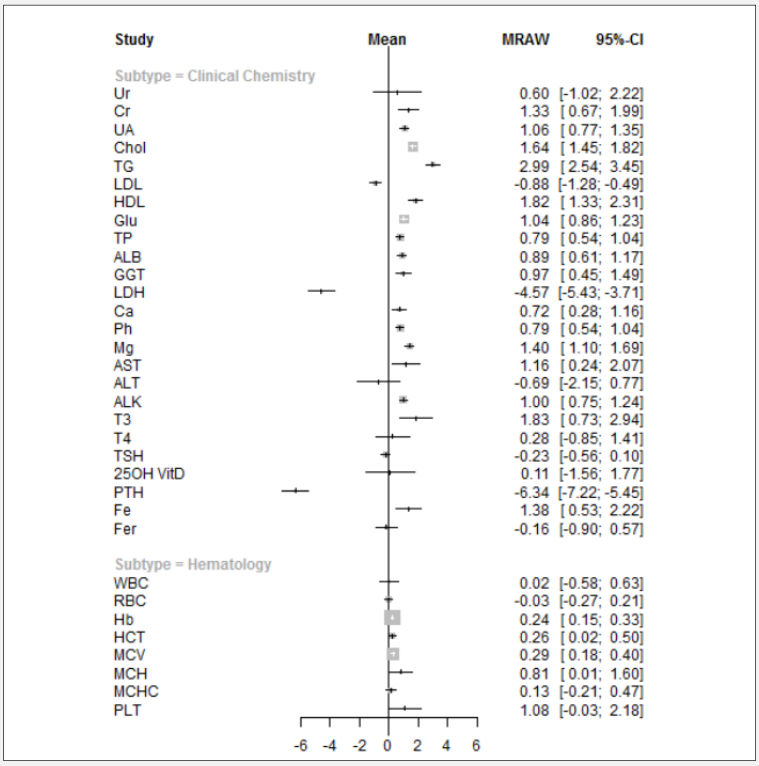


**Figure 1. Percentage change in concentration of blood parameters per 24 hours stored.**

**Squares show average percentage change and lines show 95% intervals.**

OH Vit D was observed at different time points and temperature conditions, especially at -20°C after 30 days. Elevation in TG values exceeded ACL but not TCL limits. Fortunately, glucose was stable for 30 days in frozen samples (4.6%). Although it was close to TCL and ACL (5.5% and 4.71%, respectively) but we found positive non-significant changes which can be ignored.

The positive changes might be induced by evaporation which has been reported in previous studies too. Intra-laboratory imprecision can also affect the values. For example, coefficient variation (CV%) for both T4 and 25 OH Vit D were about 7% in our laboratory which could make an obvious difference in the results. Interestingly, ALT, AST, PTH, T3, and TSH values reduced in frozen samples but only changes in ALT values was considerable (-25.5%) which is compatible with previous studies [19,20].

HbA1c results showed very little changes in both refrigerated and frozen samples which is in line of previous studies. This finding is too important for countries like Iran in which few NGSP (National Glycohemoglobin Standardization Program) certified assays still are used. However, it seems that accuracy of assays used for measurement of laboratory parameters is more important than storage condition because most analytes are stable for up to 30 days according to this study finding.

Hematological parameters including WBC, RBC, Hb, HCT, MCV, MCH, MCHC, and PLT values were stable for 48 hours at 2 - 8°C. The negative or positive changes were too small compared to both ACL and TCL limits and can be easily ignored.

## CONCLUSION

Taken together, our results demonstrate that most of the analytes are stable for a certain period of time. These findings might be useful in epidemiological studies in which the stability of samples is a great necessity.

**Declaration of Interest:**

The authors have no competing interest to declare.

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