



An Analytical Study of Retesting of Retained Sample Results

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: The sample retention policy for Clinical Chemistry analytes in accredited medical laboratories as per ISO 15189:2012 is 24 hrs. Serum/ plasma to be separated in aliquot within 20 minutes of collection unless the primary containers are gel vacutainers. Rigorous maintenance of such procedure is difficult and as a result the possibility of deviation from such schedule may not be very uncommon. The 1 year Turn Around Time (TAT) analysis of the laboratory is a good guide to find out time lag from sample collection to sample processing & average time of collecting samples in aliquot for retained sample testing. The laboratory retested 22 common analytes on the basis of such time lag and evaluated the deviation from 1st observation. The accumulated data has helped to evaluate and implement sample retention policy.

Study Design: The average time lag from collection to completion of test performance of a batch is 4hrs± 30 minutes. The analytes were retested in the time lag. After accumulation of sufficient data the time lag increased to 6 hours±30 minutes which is the average lag from sample collection to end of the day duty personnel. In the 3rd phase total retention time ie, 24 hrs has been considered as time interval of retained sample retesting. But the samples remained at room temperature for 6hrs±30minutes before being preserved at 2°C-8°C. Hence time lag was (6hrs±30min) at room temperature and 17hrs ±30min at 2°C-8°C. The samples always retested from primary container.

Place and Duration of Study: The study took place in JMD Diagnostics Private Limited, Kolkata,

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India. The duration of study is 2 yrs.

Methodology: The analytes were tested in Cobas Integra 400plus system. The tests have been performed as routine tests and considered as 1st observation. 2nd observation values obtained after the specified time lag. The results obtained were compared using statistical software. Comparison of 1st and 2nd results and bias of all analytes were studied. Electrolytes have been eliminated from the study as the electrolytes are preferred to be retested from freshly collected sample. Labile parameters like L-Lactate, ammonia, bicarbonate were also not considered for the same reason.

Conclusion: Only 3 analytes, total protein, total calcium and inorganic phosphorus cannot be preserved in primary containers. The analytes also need not to be separated within 20 minutes of collection. Upto 4hrs±30 minutes all the parameters have shown excellent correlation coefficient. Hence, the laboratory earns a time lag between collection to preservation of samples for these analytes. For other 19 analytes sample may be kept in primary container.

Keywords: Retesting; additional test; clinical biochemistry; regression co-efficient(r).

ABBREVIATIONS

TAT; CLSI; NABL.

1. INTRODUCTION

The guideline of Indian Laboratory Accreditation Body, NABL 112 [1] and CLSI [2] have certain instructions for the maintenance of quality of testing and procedures for accredited laboratories. The retention period of a sample after collection is 24 hrs [1,2]. The retained sample is preserved for additional/ repeat test on request. Preservation criteria are at 2°C-8°C for 24 hrs (from the time of collection). It has been observed that there is a time lag from sample collection to receiving time of the laboratory. From receipt of the laboratory till processing a time lapse also has been noted. The total time interval is not less than 4 hrs (4hrs±30minutes).

The data obtained from Turn Around Time (TAT) analysis of 1 year from the authors laboratory. Therefore, to maintain quality of test performance the tests need to be repeated after the lag to check the deviation from 1st result.

The optimum storage time of clinical biochemistry analytes were standardized by Marjini et al. [3], Ali L et al. [4] and the studies proved the stability of some of the analytes upto 9 days. The storage temperature was 2°C-8°C. The serum samples were separated by centrifugation in aliquot within 20 minutes of collection. But in a busy routine clinical biochemistry laboratory with approximate sample load 100/day preservation of samples in aliquot within 20 minutes of collection is difficult, rather impossible. The average time of separating serum /plasma in aliquot is 6-7hours, obtained from TAT (generally before charge handover of

laboratory personnel). So, an accredited laboratory should accept the practical situation and the deviation from 1st result after such lag should be checked.

Though after retention of 6hrs the aliquots are being preserved, the author felt a 3rd phase observation of 24 hrs may help the laboratory personnel. If the samples are kept in primary container instead preserving in aliquot and the deviation is within acceptable limit then the laboratory may validate their own preservation policy. The validation of policy saves time of pouring samples in aliquot, saves the laboratory from the personal error during the process and unnecessary procurement of microcentrifuge tubes needed to preserve the serum/plasma in aliquot. As total retention time is 24 hours so for the 3rd phase study of deviation the time lag should be 6hrs±30minutes at room temperature and at 2°C-8°C for 17hrs± 30minutes. Hence, the study of deviation is actually a study of deviation when samples are stored in primary container only.

The author decided to select 5 analytes for retesting in every month so that each parameter is being retested at least 6 times in a month giving the option of statistical calculation of deviation. Minimum deviation reflects ambience of room temperature, efficiency of the instrument and reagents and quality of the evacuated primary container. Stress has been given to tight capping of the primary container immediately after performance to prevent deviation due to evaporation of serum sample.

Such study is not essential for laboratories using gel vacutainers as gel effectively separates cells from serum. But gel vacutainers are expensive and hence only in use when requested by

patients. Moreover, if less expensive primary container can satisfy the criteria of deviation of the retention period, it should be accepted by the laboratory.

2. MATERIALS AND METHODS

2.1 Study Materials

Patient samples were selected at random. The retesting of retained sample analysis is one of the quality assurance criteria for the tests under the scope of Indian Accreditation body. So, for the study of analysis of test results patient consent is not needed. Result analysis neither discloses patient identity. So, integrity and confidentiality of patient information are protected. Samples are collected in evacuated containers with additive/no additive. Gel vacutainers are not in use.

2.2 Methods

The analytes tested are plasma glucose, serum urea, creatinine, uric acid, Total Protein, Albumin, Total Cholesterol, HDLC, LDLC, TG, AST, ALT, ALP, Amylase, Lipase, Total Calcium, Inorganic Phosphorus, CPK, LDH, GGT, Total Bilirubin and Direct Bilirubin. The tests were performed in Cobas Integra 400 Plus automated system. The objective of retained sample testing is to check repeatability performance of the laboratory. The criteria is, a single test to be repeated in the same method and system by two different laboratory personnel to eliminate personal bias. The laboratory selected 5 parameters on every month based on method mode (end point, kinetic etc). As a result for each parameter the laboratory is getting at least $5 \times 2 = 10$ observations per month per analyte. Hence after 2 years the evaluations of deviations of 22 analytes have been done. Time lag between two observations in the 1st phase was 4hrs±30minutes which is the lag between collection to completion of processing of samples. In the 2nd phase 6hrs±30 minutes, the lag of collection to the end of day duty ie, before handover of the charges. In the 3rd phase total 24hrs±60minutes(at 23°C-25.8°C for 6hrs±30minutes then 17hrs ±30 minutes at 2°C-8°C). After 24 hrs the samples are disposed as per National regulation of Waste Disposal. The lag periods obtained from TAT analysis data of the laboratory.

2.3 Statistical Calculations

The samples have been randomly chosen. The mean of 1st and 2nd observation of every analyte

in every phase is calculated. Difference from first observations and bias evaluated. Mean of the deviation for every analyte in each phase, Mean X (mean of 1st observations), Mean y (mean of 2nd observations) have been calculated. CV% have been evaluated as follows:

$$CV\% = \frac{\text{Mean of deviation} \times 100}{\text{Mean of 1}^{\text{st}} \text{ observation (all results)}}$$

Regression coefficient have also been calculated from Winks SDA – online statistical data analysis calculator. The parameters with regression coefficient 0.95-1.02 have been chosen as validated parameter for preservation in primary container.

3. RESULTS AND DISCUSSION

Results of 1st, 2nd and 3rd phase are given in Tables 1, 2 and 3.

Table 1 data confirms excellent repeatability of all analytes at above mentioned time lag.

Both CV% and regression coefficient correlated with each other [5]. Mean X and Y are mean of all 1st & 2nd observations of an analyte at a particular time lag which is necessary to calculate the regression coefficient. SD reflects the mean of deviations obtained from different patient results which is unlike of SD determination of Internal Quality Control sample where same sample is being tested and deviation is being calculated. The SD and CV% obtained from such analysis shows negligible deviation from 1st results. Deviations from 1st observation have also been observed. Zero deviation indicates same result after retained sample test, deviation (+ve) indicates raised value and deviation (-ve) low value during retesting. The deviations are taken into account to observe bias of an analyte after retaining the sample in primary container.

Table 2 data shows good CV% and Mean X, Mean Y for all analytes. But regression coefficient data [5] shows unacceptability for T Calcium (r=0.9) and T Protein (r=0.95). Hence, serum samples with request for T Calcium and protein are to be preserved in aliquot within 4hrs after collection.

Table 1. Time lag 4hrs ±30 minutes

| Parameter | Unit | Mean X | Mean Y | Dev (+ve) | Dev (-ve) | Dev(0) | SD | CV% | Regression Coefficient(r) |
|----------------|-------|--------|--------|-----------|-----------|--------|-------|------|---------------------------|
| Glucose | mg/dL | 283.71 | 283 | 5 | 7 | 2 | 3.71 | 1.3 | 1.0 |
| Urea | mg/dL | 113 | 115 | 4 | 0 | 3 | 1.8 | 1.64 | 1.0 |
| Creatinine | mg/dL | 6.14 | 6.05 | 3 | 5 | 0 | 0.101 | 1.64 | 1.0 |
| Uric acid | mg/dL | 5.96 | 6.0 | 7 | 0 | 1 | 0.06 | 1.0 | 0.98 |
| T. Cholesterol | mg/dL | 190.5 | 192 | 3 | 2 | 0 | 1 | 0.52 | 1.0 |
| HDLC | mg/dL | 73 | 73 | 2 | 1 | 2 | 0.33 | 0.48 | 1.0 |
| LDLC | mg/dL | 66.5 | 67 | 3 | 0 | 2 | 0.75 | 1.13 | 1.0 |
| Triglycerides | mg/dL | 266.5 | 270 | 4 | 4 | 0 | 3 | 1.12 | 1.0 |
| T Bilirubin | mg/dL | 3.07 | 3.04 | 3 | 2 | 0 | 0.05 | 1.64 | 1.0 |
| D Bilirubin | mg/dL | 1.954 | 1.94 | 3 | 2 | 0 | 0.032 | 1.64 | 1.0 |
| T Protein | g/dL | 7.26 | 7.26 | 1 | 1 | 3 | 0.04 | 0.55 | 0.99 |
| Albumin | g/dL | 3.96 | 3.98 | 2 | 1 | 2 | 0.06 | 1.51 | 0.99 |
| AST | U/L | 197.8 | 200.6 | 5 | 0 | 0 | 2.8 | 1.42 | 1.0 |
| ALT | U/L | 285.71 | 285.71 | 5 | 2 | 0 | 2.86 | 1.0 | 1.0 |
| ALP | U/L | 148 | 148.86 | 5 | 2 | 1 | 1 | 0.96 | 1.0 |
| GGT | U/L | 58.8 | 57.6 | 3 | 2 | 1 | 2.4 | 4.08 | 1.0 |
| Amylase | U/L | 83.5 | 83.33 | 2 | 3 | 1 | 2.5 | 2.99 | 1.0 |
| Lipase | U/L | 49 | 49.2 | 2 | 1 | 2 | 2.2 | 4.49 | 0.99 |
| T Calcium | mg/dL | 9.11 | 8.84 | 1 | 4 | 0 | 0.28 | 3.09 | 0.99 |
| I Phosphorus | mg/dL | 3.74 | 3.75 | 1 | 3 | 1 | 0.032 | 0.85 | 1.0 |
| CPK | U/L | 389.8 | 385.6 | 2 | 2 | 1 | 5.8 | 1.49 | 1.0 |
| LDH | U/L | 305.8 | 305.6 | 3 | 2 | 0 | 3.8 | 1.24 | 1.0 |

Table 2. Time lag 6hrs ±30 minutes

| Parameter | Unit | Mean X | Mean Y | Dev (+ve) | Dev (-ve) | Dev(0) | SD | CV% | Regression Coefficient(r) |
|----------------|-------|--------|--------|-----------|-----------|--------|-------|------|---------------------------|
| Glucose | mg/dL | 197 | 198 | 2 | 2 | 2 | 1.33 | 0.67 | 1.0 |
| Urea | mg/dL | 52.83 | 53 | 3 | 2 | 1 | 1.5 | 2.84 | 1.0 |
| Creatinine | mg/dL | 1.3 | 1.35 | 1 | 5 | 0 | 0.05 | 3.84 | 1.0 |
| Uric acid | mg/dL | 7.69 | 7.67 | 4 | 2 | 0 | 0.095 | 1.23 | 1.0 |
| T. Cholesterol | mg/dL | 171.4 | 174 | 3 | 1 | 1 | 3 | 1.75 | 1.0 |
| HDLC | mg/dL | 46 | 46 | 0 | 2 | 3 | 0.4 | 0.87 | 1.0 |
| LDLC | mg/dL | 106.16 | 108 | 3 | 0 | 3 | 1.66 | 1.56 | 1.0 |
| Triglycerides | mg/dL | 181.6 | 184 | 4 | 1 | 0 | 3.6 | 1.98 | 1.0 |
| T Bilirubin | mg/dL | 4.04 | 3.97 | 2 | 5 | 1 | 0.07 | 1.71 | 1.0 |
| D Bilirubin | mg/dL | 0.674 | 0.655 | 2 | 5 | 1 | 0.02 | 2.97 | 1.0 |
| T Protein | g/dL | 7.33 | 7.46 | 5 | 1 | 1 | 0.13 | 1.77 | 0.95 |
| Albumin | g/dL | 4.58 | 4.61 | 2 | 0 | 4 | 0.033 | 0.72 | 1.0 |
| AST | U/L | 25.43 | 26 | 2 | 1 | 4 | 0.86 | 3.38 | 0.98 |
| ALT | U/L | 31.94 | 32 | 2 | 1 | 4 | 0.34 | 1.06 | 1.0 |
| ALP | U/L | 98 | 100 | 7 | 0 | 0 | 2 | 2.04 | 1.0 |
| GGT | U/L | 362.66 | 368.53 | 4 | 2 | 0 | 6.86 | 1.89 | 1.0 |
| Amylase | U/L | 51.6 | 51.14 | 2 | 2 | 3 | 1.86 | 3.6 | 1.0 |
| Lipase | U/L | 49.14 | 50.2 | 4 | 0 | 1 | 0.86 | 1.75 | 1.0 |
| T Calcium | mg/dL | 8.88 | 8.85 | 4 | 2 | 0 | 0.33 | 3.71 | 0.9 |
| I Phosphorus | mg/dL | 4.97 | 5.02 | 4 | 0 | 1 | 0.078 | 1.57 | 1.0 |
| CPK | U/L | 1383 | 1400 | 3 | 3 | 0 | 19 | 1.38 | 1.0 |
| LDH | U/L | 211 | 212 | 4 | 2 | 0 | 3.33 | 1.58 | 1.0 |

After 24 hrs it is being observed that though CV% is acceptable in all 22 analytes, regression coefficients of TProtein, Inorganic Phosphorus and T Calcium are not within the acceptable limit.

So, 19 analytes may be preserved in primary container. Only above mentioned 3 parameters to be preserved in aliquot within 4 hrs±30 minutes of collection.

Table 3. Time lag 6hrs ±30 minutes at room temperature and 17hrs±30 minutes at 2°C -8°C

| Parameter | Unit | Mean X | Mean Y | Dev (+ve) | Dev (-ve) | Dev(0) | SD | CV% | Regression coefficient(r) |
|----------------|-------|--------|--------|-----------|-----------|--------|--------|------|---------------------------|
| Glucose | mg/dL | 199 | 199 | 4 | 4 | 5 | 1.71 | 0.86 | 1.0 |
| Urea | mg/dL | 45.11 | 47 | 8 | 1 | 0 | 3 | 6.65 | 1.0 |
| Creatinine | mg/dL | 1.92 | 1.96 | 4 | 3 | 1 | 0.068 | 3.54 | 1.0 |
| Uric acid | mg/dL | 6.59 | 6.5 | 6 | 6 | 0 | 0.161 | 2.44 | 1.0 |
| T. Cholesterol | mg/dL | 175.6 | 178 | 12 | 1 | 2 | 2.86 | 1.63 | 1.0 |
| HDLC | mg/dL | 35.2 | 36 | 5 | 0 | 0 | 1.2 | 3.41 | 0.99 |
| LDLC | mg/dL | 106.2 | 105 | 3 | 1 | 1 | 3.4 | 3.2 | 0.99 |
| Triglycerides | mg/dL | 150.66 | 153 | 8 | 2 | 2 | 2.66 | 1.76 | 1.0 |
| T Bilirubin | mg/dL | 1.46 | 1.46 | 5 | 7 | 0 | 0.04 | 2.67 | 1.0 |
| D Bilirubin | mg/dL | 0.253 | 0.248 | 4 | 6 | 2 | 0.0116 | 4.58 | 0.99 |
| T Protein | g/dL | 7.15 | 7.13 | 3 | 4 | 1 | 0.2 | 2.79 | 0.91 |
| Albumin | g/dL | 4.42 | 4.37 | 2 | 2 | 2 | 0.08 | 1.81 | 0.99 |
| AST | U/L | 23.2 | 24.27 | 10 | 1 | 0 | 1.27 | 5.47 | 0.98 |
| ALT | U/L | 67.09 | 66.72 | 4 | 4 | 3 | 1.82 | 2.71 | 1.0 |
| ALP | U/L | 114.22 | 116.55 | 7 | 1 | 1 | 2.33 | 2.04 | 1.0 |
| GGT | U/L | 105.62 | 108.37 | 5 | 3 | 0 | 4 | 3.94 | 1.0 |
| Amylase | U/L | 84.86 | 91 | 6 | 0 | 1 | 2.74 | 3.23 | 1.0 |
| Lipase | U/L | 30.6 | 31.4 | 2 | 0 | 3 | 1.2 | 3.92 | 0.98 |
| T Calcium | mg/dL | 9.93 | 9.81 | 3 | 4 | 0 | 0.34 | 3.42 | 0.71 |
| I Phosphorus | mg/dL | 3.2 | 3.54 | 9 | 0 | 0 | 0.115 | 3.59 | 0.9 |
| CPK | U/L | 935.28 | 809.28 | 4 | 3 | 0 | 42.37 | 4.53 | 1.0 |
| LDH | U/L | 229.33 | 210 | 2 | 4 | 0 | 6.4 | 2.79 | 0.99 |

The pictorial presentation of all the parameters (Fig. 1) presents results of 1st and 2nd observation. Results of 1st performance are along Y-axis and repeat test results are along X-axis. The pictorial presentation has shown retesting results of retained samples of all three lag periods. Graphical presentations (Fig. 1) of three separate lag periods for 22 parameters were not given to prevent unnecessary occupancy of space. As the results have shown good regression correlation the same was not felt to be necessary.

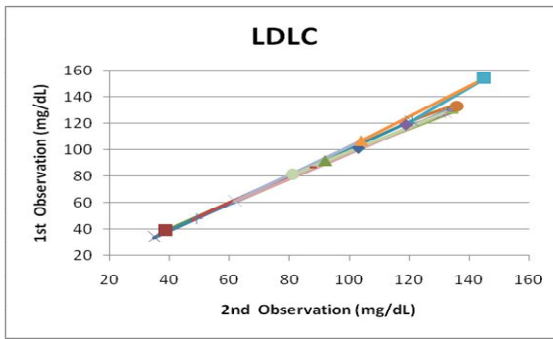
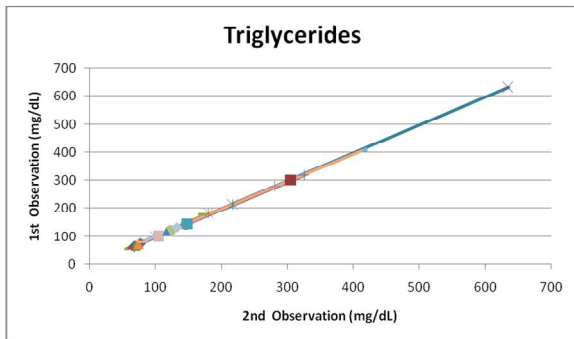
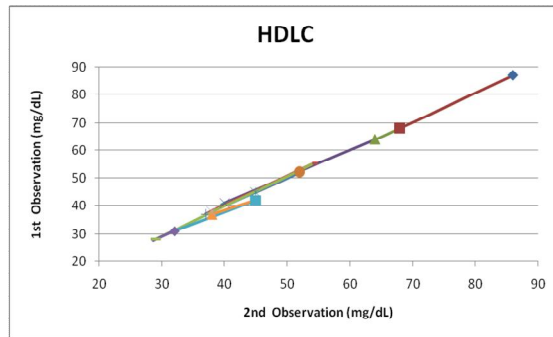
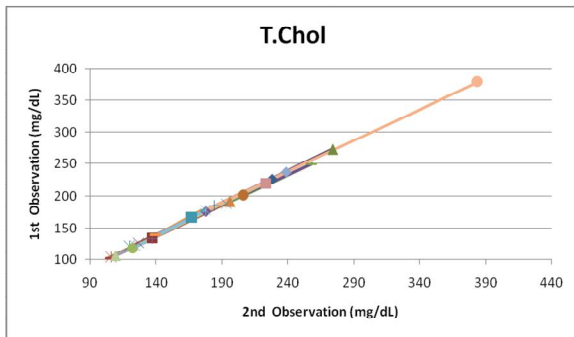
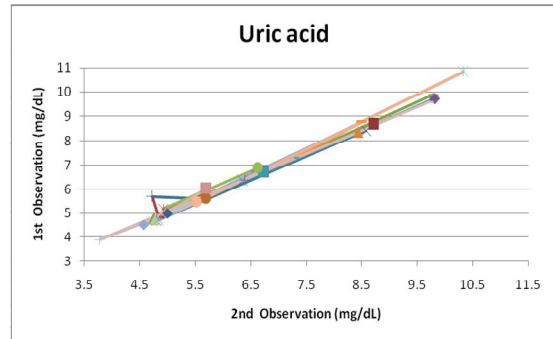
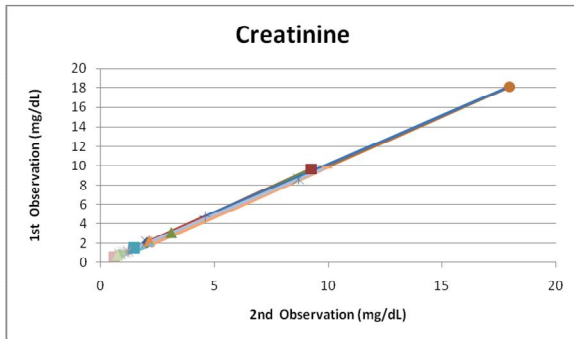
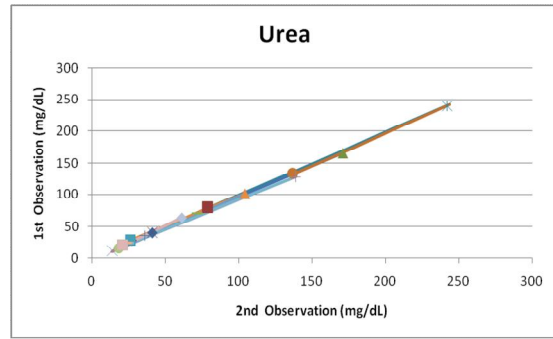
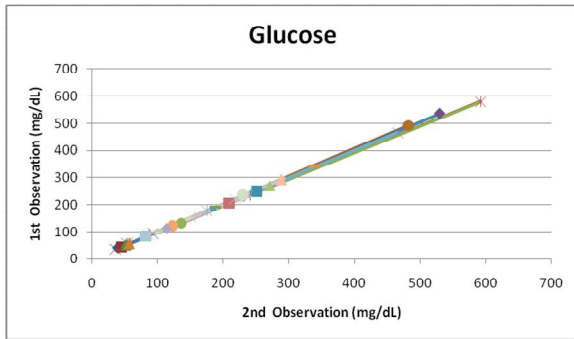
Previous observations regarding storage time of analytes are all recent. Samples stored at -20°C and Q-probe study showed good repeatability after 7 days for 19 analytes [4,6]. The observations were excellent but not relevant in the present scenario. The samples were preserved within 20 minutes of collection. But the objective of present study is to determine maximum retention time of samples in primary container.

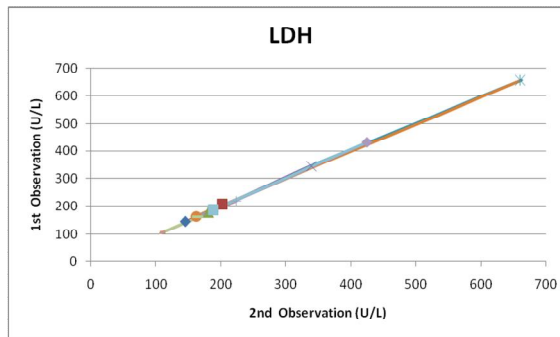
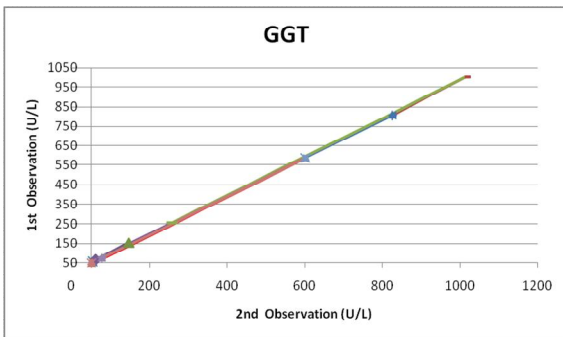
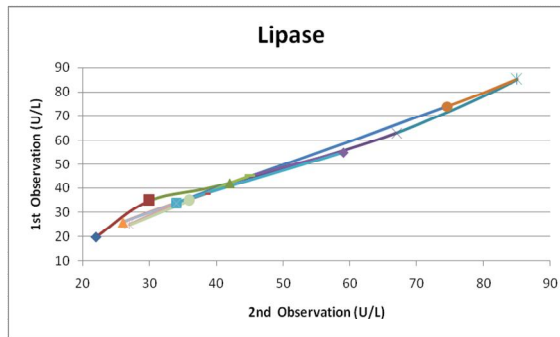
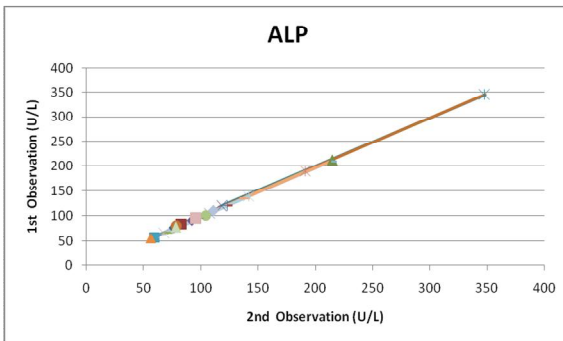
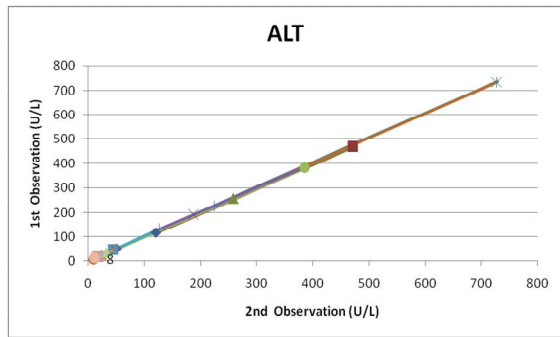
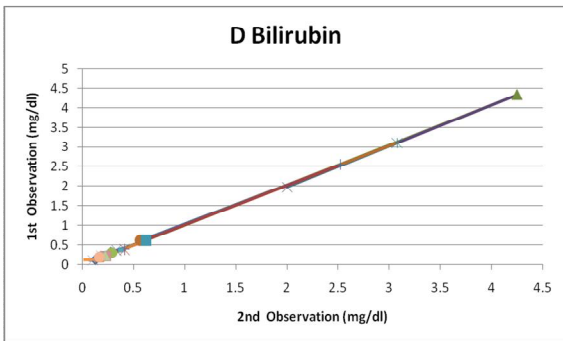
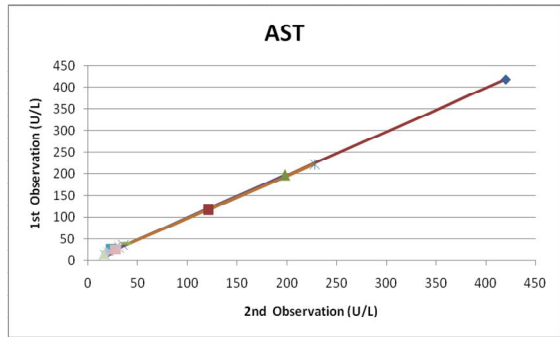
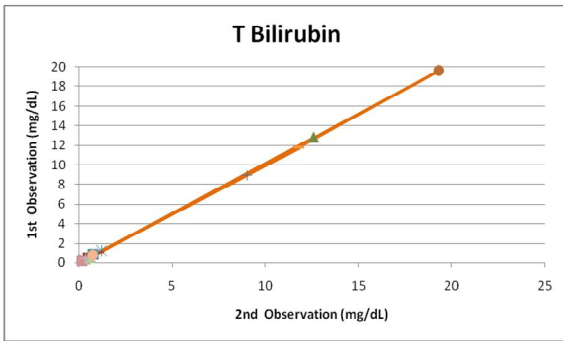
The study reflects efficiency of the automated system, reagents, ambience of room temperature, quality of primary sample container, efficient maintenance of TAT and competence of laboratory personnel. Deviation from any of such factors is suggestive of re- evaluation. The CV%

of all 22 analytes show deviations which are within clinically acceptable limit but the laboratory decided to follow the correlation coefficient. The laboratory decided to find out bias for every analytes and the distribution patterns are shown in Fig. 2.

The distribution data analysis (Tables 1, 2, 3, Fig. 2) gives the impression of bias of an analyte. Regression and bias curves of all analytes were done after accumulation of all lag period values.

Evenly distributed data in all lag periods have been observed in analytes like glucose, creatinine, HDLC, LDLC, albumin, ALT, amylase, total calcium. Maximum number of zero bias observation has been found out in LDH, Lipase, Albumin, CPK, HDLC & LDLC. Consistent positive bias have been observed in analytes like uric acid, total cholesterol, triglycerides, total protein, AST, ALP and Inorganic phosphorus. Lipase has shown only zero and positive bias. Negative bias have been observed in total and direct bilirubin from 2nd phase. Such bias are system bias. In urea and amylase even distribution pattern have been observed at the 1st phase then positive bias at the 2nd and 3rd phase from which it may be concluded that the bias for these analytes are directly proportional to time.





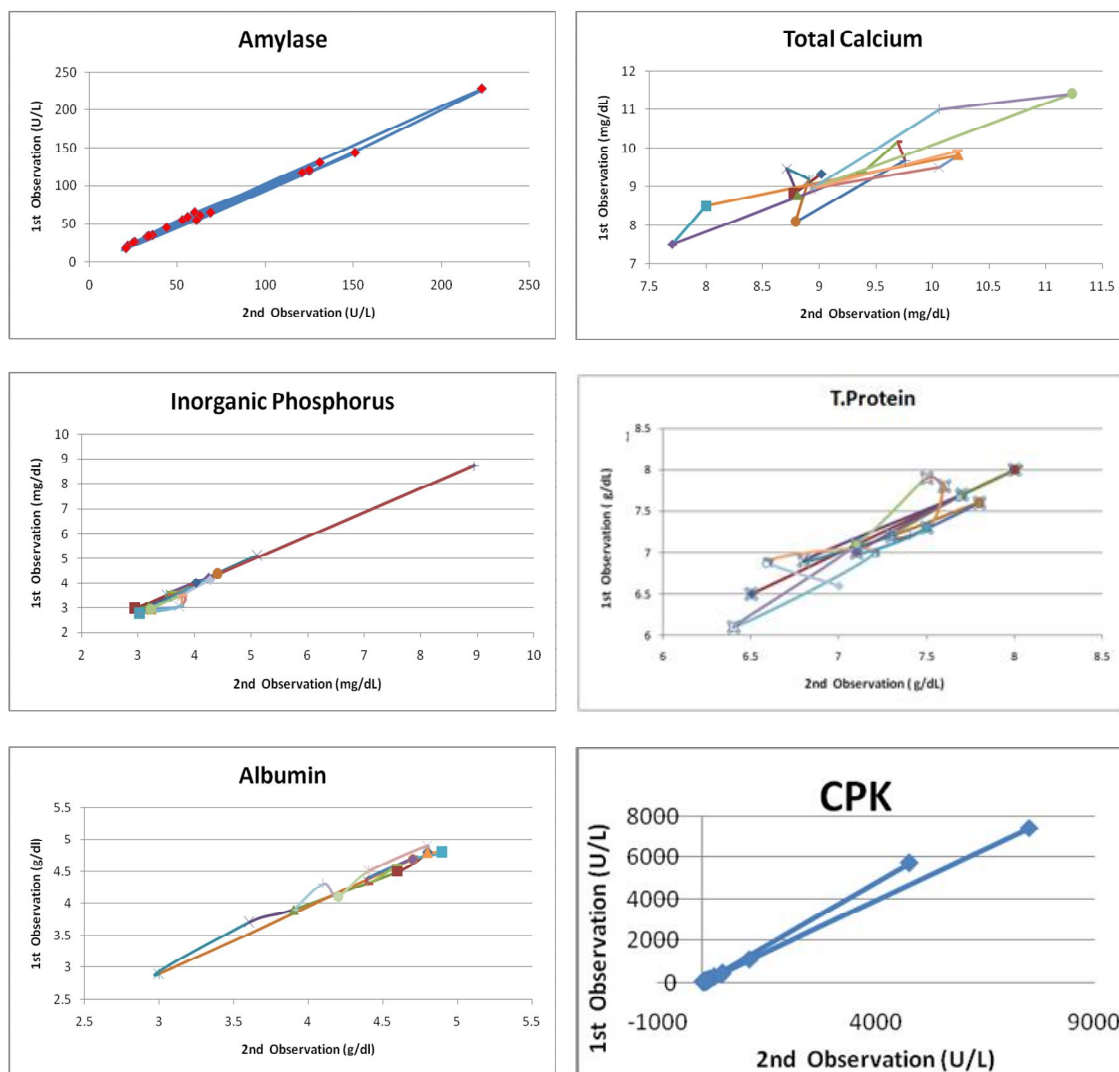


Fig. 1. Retesting of retained sample

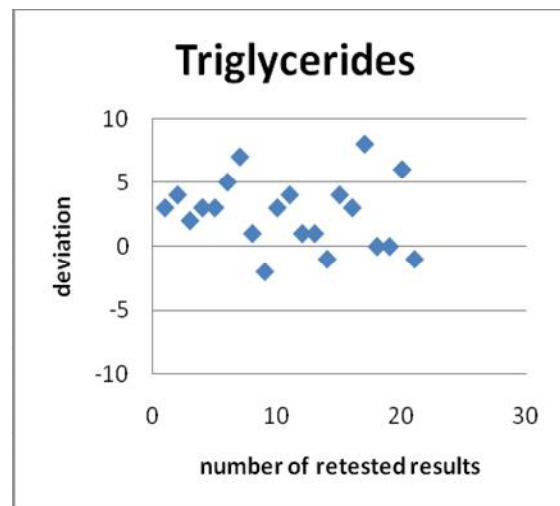
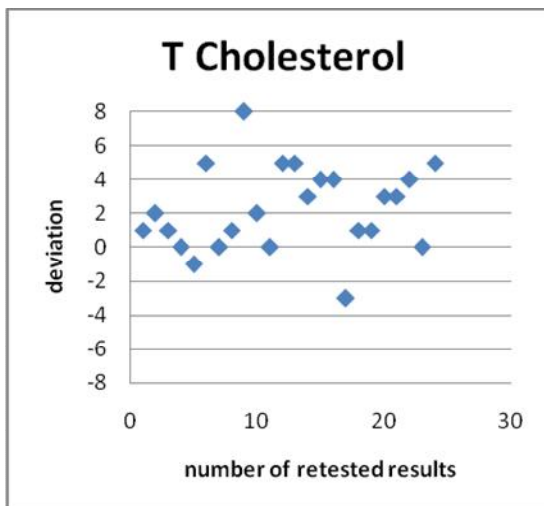
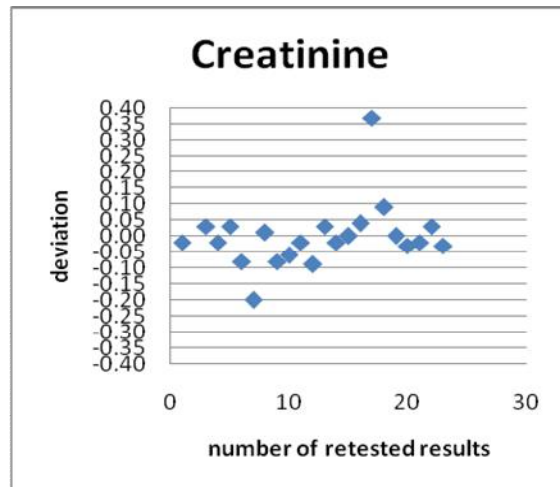
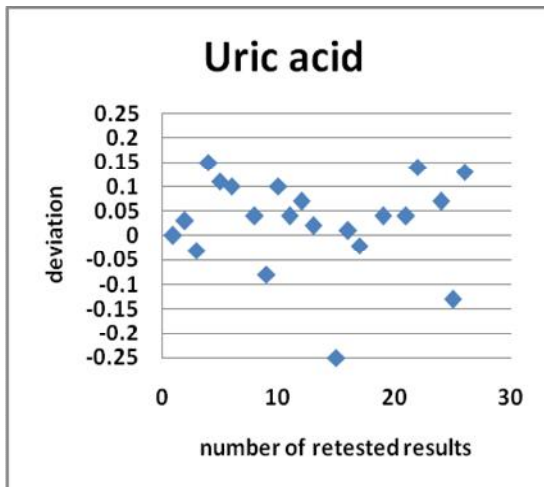
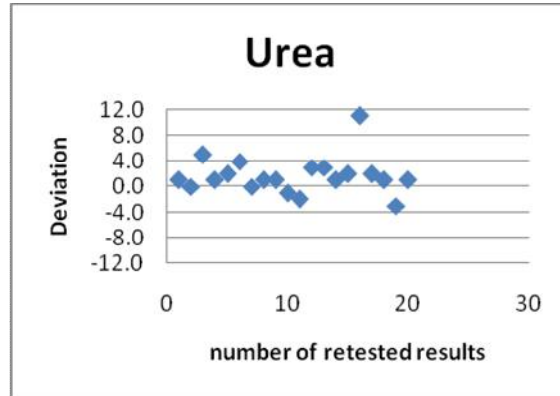
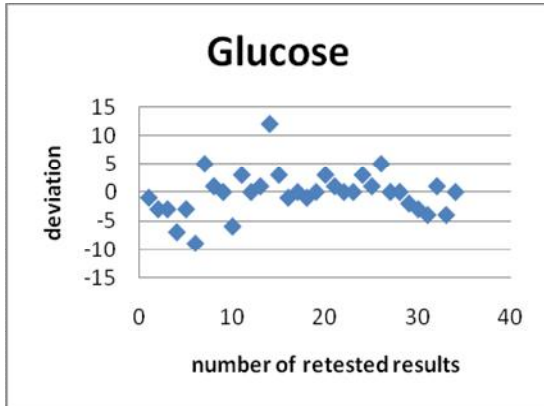
Previous works suggested that stability have been observed for 25 analytes (included electrolytes also) at 25°C in human serum upto 48 hrs [6,7]. Serial study at 4°C, 22°C, -20°C for 48hrs to 14 days were performed showing stability for such a long period [6,7]. Differences in data were stated to be negligible. Only one study mentioned increase in inorganic phosphorus, uric acid, HDLC, triglycerides and decrease in bilirubin total and direct, CPK, AST, LDLC when stored at room temperature [8]. The storage time was 48hours to 14 days. The study included electrolytes also but as per accreditation guideline electrolytes are preferably to be performed from fresh collection as contact with RBC raises potassium value. Present observations are in accordance with previous

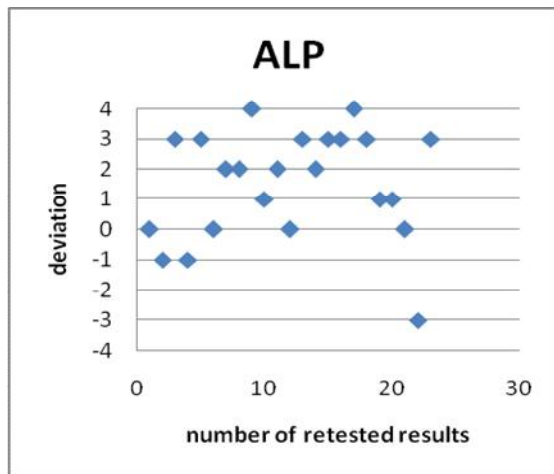
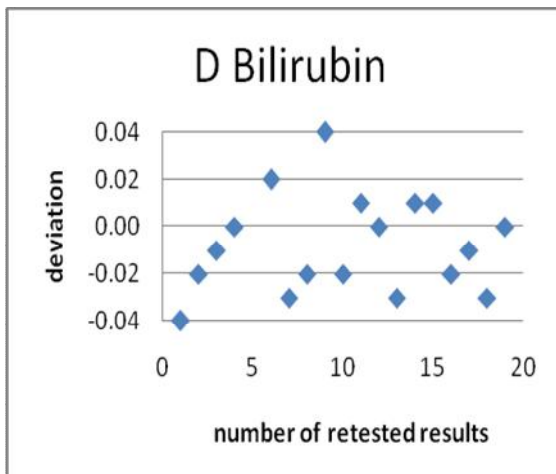
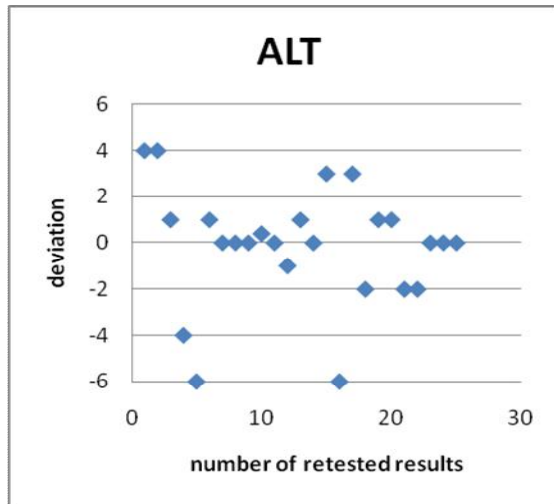
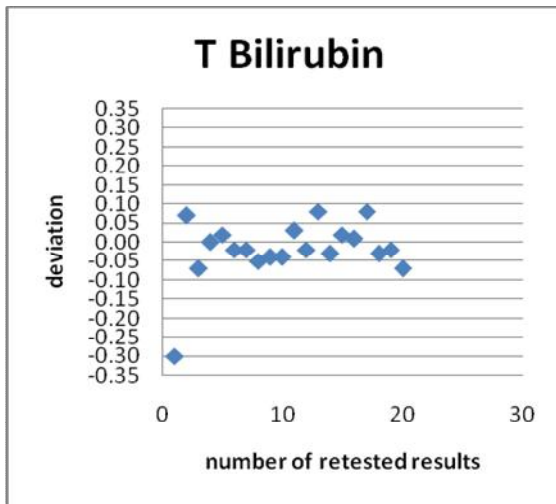
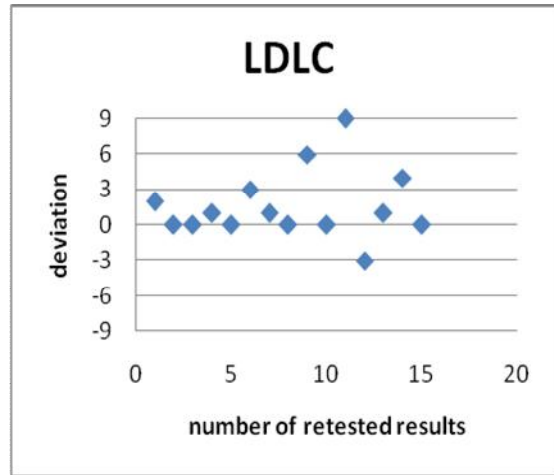
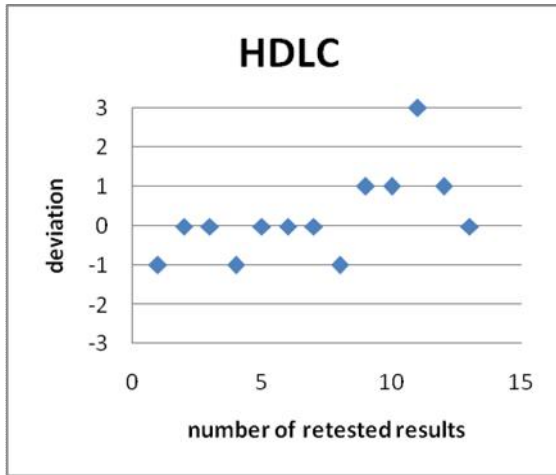
observation for some analytes like uric acid, triglycerides and bilirubin total and direct.

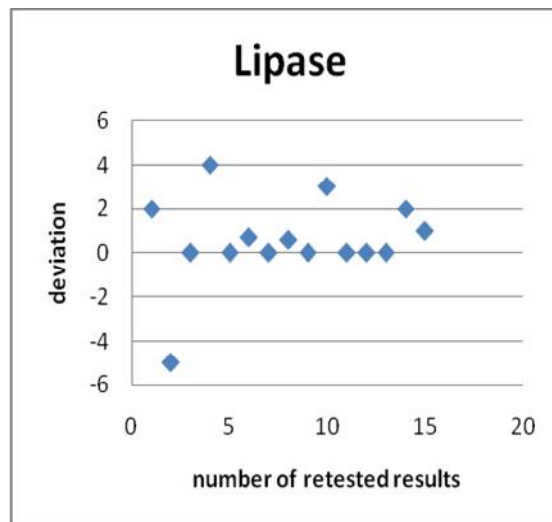
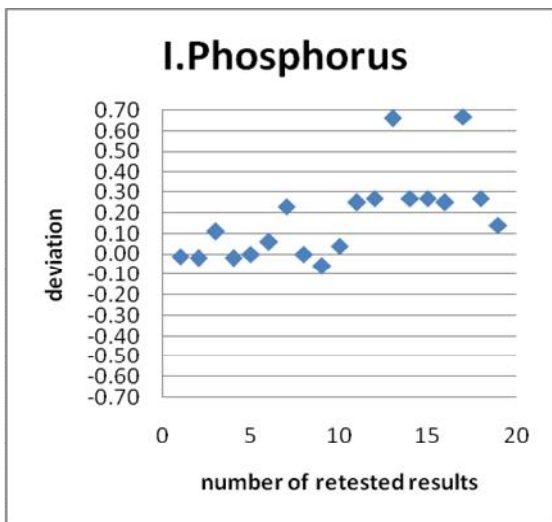
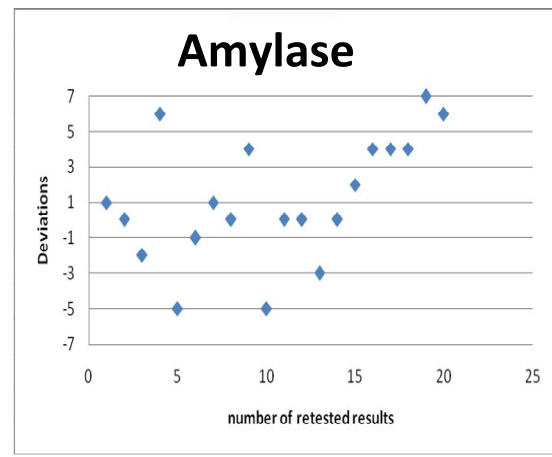
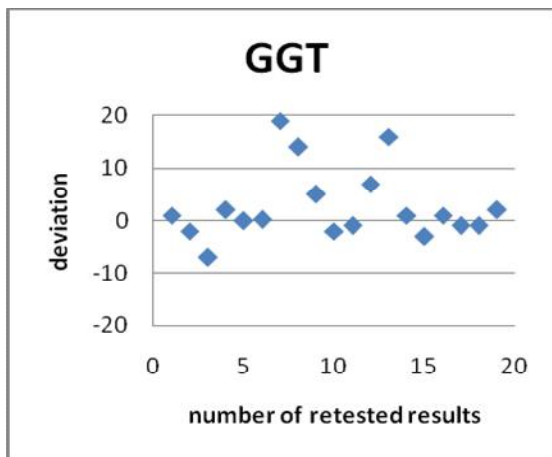
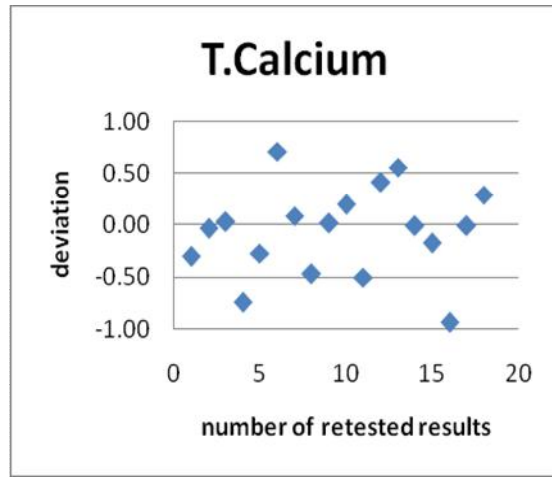
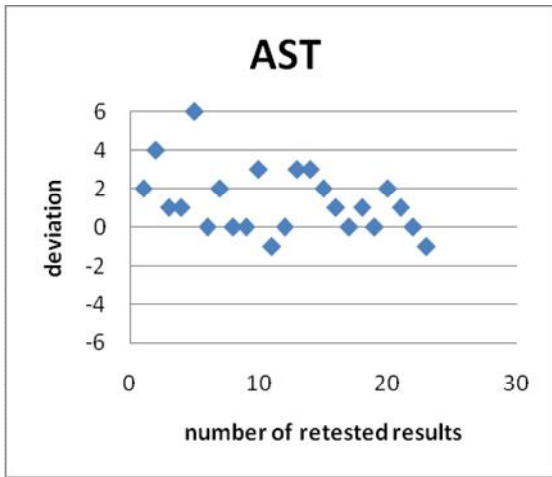
The important deductions from this study are, 90% of routine samples do not need to be kept in aliquot if the retention time is 24 hours which is important from both Human resources and financial point of views. Total protein results get affected because it is the base of sample matrix. Hence, slightest change in sample volume affects the correlation coefficient. Methods of estimation of total calcium and phosphorus are very sensitive and failure to satisfy the criteria of correlation coefficient may be attributed to that. Finally, the CV% of all analytes are satisfactory but correlation coefficient criteria made three parameters unacceptable for such preservation.

So, for similar study, correlation coefficient to be considered as the determinant instead CV%. The study is specifically useful for paediatric samples

as collection & preservation of paediatric samples are difficult.







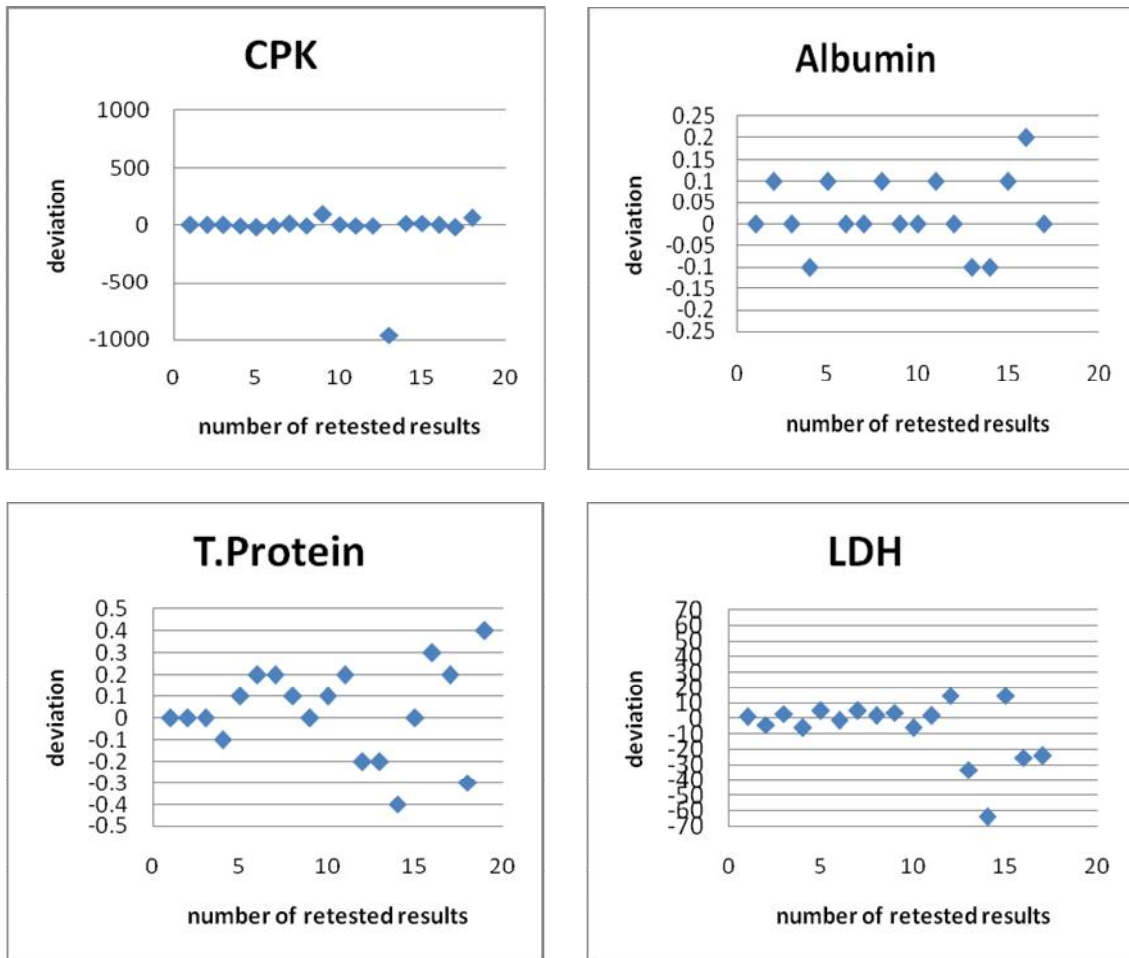


Fig. 2. Bias evaluation

4. CONCLUSION

The validation study may be used as a guideline for sample preservation. But the validity is dependent on certain factors like laboratory environment, time lag between collection and receipt by the laboratory, instrument, reagents, competence of laboratory personnel and quality of primary container. Any deviation from such factor/factors needs re evaluation. The presenting authors laboratory has decided to continue this study as part of quality control procedure so that any deviation may be noted and corrective and preventive action may be implemented.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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2. The laboratory personnel - JMD Diagnostics Private Limited

COMPETING INTERESTS

Author has declared that no competing interests exist.

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