Effect of sample storage and time delay (delayed processing) on analysis of common clinical biochemical parameters

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Abstract

Introduction: The objective of the study was to studying the effect of delay in time on biochemical estimations, the effect of ambient temperature on assays and the effect of hemolysis on certain commonly requisitioned parameters in serum – includes glucose, urea, creatinine and electrolytes and enzyme Alkaline phosphatase.

Materials and Method: Study includes 53 randomly selected non-fasting venous blood samples as advice by their clinicians for routine biochemical assays were taken for three formats of which includes biochemical estimations done after 4hr delay at room temperature, 12 hrs delay at 2-8deg C, of the study on hemolysed sample at room temperature.

Results: Statistical analysis were done using Spss 13.0 version, the results were expressed in mean showed significant alteration of serum glucose and Alkaline phosphatase after 4hours at room temperature (p=0.00; 0.017), serum glucose and, creatinine and ALP after 12 hours delay at 2-8deg C. (p=0.00; 0.00; 0.043) respectively.

Conclusion: This study may be useful to help define acceptable delay times and storage conditions when a short time between sample collection and processing is not possible.

Keywords: Pre analytical, Hemolysis

Introduction

In the era of evidence based medicine, physician uses the laboratory report for assistance in diagnosis and management of the patients to confirm a clinical impression. An adequate understanding of each of the steps involved in the process enables the laboratorian to achieve nearly optimal conditions and consequently to improve the accuracy and precision of each measurement. Though there are many aspects which contribute to test results in the biochemistry laboratory.

The system involves several steps starting from preparation of the patient, collection of the samples, processing of the samples, estimation by auto analyzer and manual methods, reporting and interpretation of the values. Proper documentation of the samples with details of the patients is of critical importance and is implicit in the process. Hence the entire process can be viewed as including factors which are – Pre analytical, Analytical and Post analytical.[1] A close study of the above parameters of assay will ensure pinpoint-trouble shooting which is critical for good laboratory practice.

The pre analytical variables fall two categories those that are controllable and non-controllable, the controllable variables (e.g.) specimen collection, tables, diet, life style, drug intake etc. and non-controllable variables (e.g. age, gender, race etc.).

Errors in pre analytical, analytical and post analytical practices account for 32 – 75% of laboratory errors [2] and span the time from when the test is ordered by the physician until the sample in ready for analysis.

This present study was aimed to study the effects of differing practices in processing and storage conditions on the stability of various clinical chemistry parameters in serum samples.

The objectives of the study were
1. To study the effect of delayed serum separation on stability of commonly assayed clinical chemistry parameters like Glucose, Urea, Creatinine and Alkaline phosphatase level.
2. To study the effect of storage at different temperatures on stability of reagents and common biochemical analytes in serum.
3. To study the effect of hemolysis on the levels of serum electrolytes.

Materials and Method

200 patients came for routine biochemical assays in our lab at Employees State Insurance & Post graduate Institute of Medical Education and Research, Manicktala, from that 55 randomly selected venous blood samples collected and transferred to Polypropylene tube with and without anticoagulant were enrolled in the 3 study groups.

Sample storage was done at room temperature and cold storage temperature ranging between 2–8°C. Ethical committee approvals was obtained from the institution.

Samples were divided in to three study groups.

Study group 1: Blood collected from each individual was separated after 1 hour and analyzed to work as control baseline value. The left out sample was kept at

room temperature (30-35 ºC) for 4 hrs. At the end of 4 hours left-out serum samples were reanalyzed.

Study group 2: Blood collected from each individual was allowed to clot and the serum was separated and assayed after 1hour to work as control baseline value (1hr). After analysis the left out sample was kept at 2-8ºC for 12 hours. At the end of 12 hours left-out serum samples were reanalyzed.

Study group 3: Comparing the effect of erythrocyte lysis on serum electrolytes will help us in evaluating the results of lysed samples which will prevent us improper patient care. For this the blood collected for the analysis were checked for sample lysis and the electrolyte levels were compared between the lysed and unlysed samples.

Clinical analytes assayed: Glucose estimated by GOD POD (Glucose Oxidase– Peroxidase) method, urea estimated by (urease / GLDH) method, serum creatinine estimated by modified Jaffe's method respectively were

estimated using reagent kits from Mindray Diagnostics adapted to Mindray autoanalyser and Alkaline phosphatase estimated enzymatically by (IFCC) using the reagent kit from (Mindray diagnostic – India) adapted to Mindray autoanalyser. Sodium and Potassium estimated using Ion Selective Electrodes (ISE) Technology, adapted to Siemens electrolyte analyzer.

To eliminate run-to-run analytical imprecision QC samples were before and start of each batch of assay.

Statistical analysis: Data were analyzed using the statistical software SPSS 13.0(SPSS Inc. Chicago, IL, USA).The results were expressed as Mean values. Comparison of difference between the analytes before and after storage was done using students paired t test. Comparison of difference between different groups was done using Student's' test. A p-value less than 0.05 were considered significant.

Results

Effect of temperature on stability of serum analytes

Table 1: Changes in the mean value of serum analytes on 4 hrs delayed sample when stored at room temperature

Sl. No.	Analytes	Sample size	After 1 hr	After 4hr	p value
1	Glucose (mg/dl	25	108	94	0.00**
2	Urea (mg/dl)	25	24	22	0.56
3	Creatinine mg/dl)	25	0.95	1.0	0.12
4	ALP (IU/L)	25	68	72	0.017**

*p value <0.05; **p < 0.01

As given in Table 1 shows the significant alterations in the values of glucose and Aspartate transaminase when there was delay of 4hours in estimation.

Table 2: Change in the mean value of serum analytes 12hr after storage at 2-8°C

Sl. No.	Analytes	Sample size	After 1 hr	After 12hr	p value
1	Glucose (mg/dl	25	92	77	0.00**
2	Urea (mg/dl)	25	34	35	0.56
3	Creatinine mg/dl)	25	1.2	0.9	0.12
4	ALP (IU/L)	25	73	67	0.017**

*p value <0.05; **p < 0.01

Table 2 shows the significant alterations in the values of glucose, creatinine and Aspartate transaminase activity when there was delay of 12 hours in estimation after storage at 2-8deg C.

Table 3: Change in the mean value of serum electrolyses after hemolysis

Sl. No.	Analytes	Sample size	Unlysed samples	Lysed samples	p value
1	Sodium (mg/dl)	10	134	75	0.00**
2	Potassium (mg/dl)	10	3.7	6.6	0.00**

**p < 0.01

Table 3 shows the significant alterations in the values of serum electrolytes on hemolysis.

Changes in the mean value of serum analytes on 4 hrs delayed sample when stored at room temperature in
Study group 1: Table 1. Shows the significant alterations in the values of glucose and Alkaline phosphatase when there was delay of 4hours in estimation. Glucose and Alkaline phosphatase levels were decreased after 4 hrs of storage at room temperature. No significant difference was seen in urea and creatinine levels.
Study group 2: Change in the mean value of serum analytes 12hr after storage at 2-8°C given in Table 2 shows the significant alterations in the values of glucose, creatinine and Alkaline phosphatase. Glucose and Alkaline

phosphatase levels were decreased after 12 hrs of storage at 2-8°C temperature. No significant difference was seen in urea and creatinine levels.

Study group 3: Change in the mean value of serum analytes 12hr after storage at 2-8°C was observed as given in Table 3. Shows the significant alterations in the values of serum electrolytes on hemolysis. Compared to the unlysed samples the hemolysed samples showed significant decrease in values of sodium and potassium.

Discussion

Any health care professionals or laboratory staff member knows that collection and handling are done monotonously and often without care. Owing to pressure of work in a large hospital like ours, improper procedures may be adopted. The worrisome aspects of improper sample processing on laboratory results are due to variable errors occurring in a laboratory process. Laboratory errors will lead to either repeated specimen collection for laboratory tests, or repeated laboratory analysis thus resulting in an unjustified increase in costs. Several previous studies showed variable changes in laboratory results, occurring due to poor laboratory practices and control measures.[3] 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 we investigated the effects of delay in assays after separation on some commonly assayed biochemical parameters. The possibility of using cheaper and feasible means to control these preanalytical variables.

Variation in serum analyte stability: A 4 hr delay in separation of serum caused variability in serum analyte values. ALP showed an increased in activity. This may be due to denaturation induced alteration of protein structure following by renaturation.[4,5,6] Creatinine and urea did not show any significant change even after 4 hours delay this can be attributed to the stability of the analytes i.e. non utilization of the analyte by either enzymatic means or nonenzymatic means like auto oxidation. Glucose levels decreased after 4 hours which can be due to its utilization by glycolysis.[7,8] The decrease of glucose concentration during storage may be related to sensitivity of glucose to temperature variations.

The measurement and interpretation of glucose concentrations is an area of much confusion. Plasma glucose is about 12% greater than that of whole blood because plasma has higher water content as it contains no red blood cells. In the fasting state there is little difference between arterial, capillary and venous glucose concentrations but after carbohydrate intake glucose concentration in arterial and capillary samples can exceed those of venous samples by as much as 1.8

mmol/l.[9] This is of particular practical importance in the interpretation of oral glucose tolerance a test.

For the measurement of glucose a specimen containing sodium fluoride to inhibit glycolysis and stabilize the glucose concentration is preferred. If blood is collected into a tube without preservative, glucose concentration can decrease by as much as 7% each hour as a result of glycolysis.[10] Clinically significant decreases in glucose concentration have been reported even with use of preservative.[11] If a tube without preservative is used and delay in transit to the laboratory is anticipated, storage at 4°C is preferable

Effect of temperature on the stability of serum analytes

Storage Temperature: Environmental factors like Ambient Temperature, Relative Humidity, and Air flow can affect serum analyte value.[12] Previous studies on the effect of temperature on serum analyte stability showed that majority of the analyte show detoriation at Room temperature and at lower temperature (2-8°C) overall metabolism taking place inside RBC is reduced thereby preventing the loss of serum analytes.[13,14] So, storing samples at 2–8°C was ideal for all analyte except K+. As K+ rises due to decrease in glycolysis and increase in diffusion also may be due to invisible hemolysis.[13,14] In our study storage at 2-8°C for 12 hours showed a significant decrease in all the analytes except for urea which is in contrast to previous studies this could be due to sample dilution as the samples are kept in open tubes in the refrigerator for a longer duration i.e. for 12 hours.[12]

Sample lysis: Serum electrolytes are the most common analytes requested in the clinical biochemistry laboratory. It is a standard practice to process the serum immediately after the blood specimens reach the laboratory and proceed with the assay. Sometimes the samples arrival in the laboratory is delayed due to transport from the collection centre to the central lab. The analysis may also be delayed due to the increased work load or the casual attitude of the technicians. The adverse effects of prolonged serum-clot contact time were known long back and immediate separation of the serum from cells was advised. During a prolonged serum clot contact time, both the biological activity of the cells and transmembrane diffusion can change the concentration of serum electrolytes. The current recommendation of an acceptable time interval between sample collection and serum separation is 2 hours.[15]

Information on the stability of serum electrolytes during storage of serum is often incomplete and sometimes contradictory. Donnelly et al., Who investigated the stability of 25 analytes, showed that sodium, potassium and chloride remain stable for 24 hrs at room temperature, 4°C, and -20°C.[16] Heins et al., who performed stability studies on 22 serum analytes,

found that electrolytes remain stable even after 24 hrs.[17] According to Rashmi Rasi Datta et al., stability of electrolytes (sodium and potassium) is not altered when samples are stored at 23oC. The maximum clot contact time which has no effect on the stability of electrolytes is 3 hrs.[18] However, according to Baruah A et al., samples for electrolytes should be analyzed within 1-2 hrs of centrifugation and if there is any delay in analysis, the sample should be stored under proper condition.[19]

In our study the serum sodium levels were significantly decreased in lysed samples whereas the serum potassium levels were increased in lysed samples compared with normal samples. The decrease in sodium levels could be due to sample dilution following lysis i.e. due to the release of intracellular contents of the erythrocytes. The increase in serum potassium levels in lysed samples could be explained by the high intracellular content of potassium. The percentage change in potassium is greatest at 4 °C because the lower temperature induced inhibition of Na–K ATPase pump that leads to increased release of potassium from cells.

Conclusion

Proper storage temperature and times must be considered for these analytes 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.

Limitations

Because of practical and economic constraints, only a relatively small number of specimens could be tested per analyte. A profound influence of open tubes on serum analyte levels. The run-to-run variations occurring during analyzing sample was not accounted. Samples were not run in replicates check for reproducibility.

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