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Effect of freezing and thawing on serum bilirubin and total protein

Biochemistry

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ABSTRACT

In a clinical laboratory test done on daily basis to measure the concentration of a constituent in a body fluid in order to give information relevant to the clinical state of a patient. To withstand the stability of the constituent or analytes in a body fluid for a period of time when the sample is stored under various storage conditions is always meant to be challengeable. This present study has been performed on two important biochemistry analytes to study the effect of freezing and thawing on the analytes. 20 Left over serum samples obtained from the free laboratory of Biochemistry were analyzed on day 1 for baseline measurement for both the analytes followed by freezing the sample at -20˚c and thawing at room temperature. In such manner the samples were made to undergo 10 freeze and thaw cycles. The results were compared with those obtained on the first day. Mean, standard deviation, and percentage difference were evaluated statistically. The Comparison of the data showed mean change by 0.03gm/dL from day1 to day10 and for total bilirubin mean change by 0.18mg/dL from day1 to day 10 with decrease in the total bilirubin values by 4.08% and total protein by 0.76%. As a result the study concludes that total bilirubin values gradually decrease when we repeatedly freeze at - 20˚c and thaw at room temperature. Total protein is stable for 10 cycles of freezing and thawing. Hence we need to be cautious while planning the studies and research projects which take up bilirubin as the parameter where repeated freezing and thawing is required.

KEYWORDS:

Total bilirubin, Total protein, Freeze & Thaw cycle, Stability, Jaundice

Introduction:

Laboratory test are used by the clinicians for diagnosis, monitoring and prognosis in patients with different disease [1]. The problem faced by clinical laboratories is the stability of serum analytes [2], were change in the result of the values of analytes from baseline concentration of the fresh sample due to different temperature and time [3]. Many investigators have studied changes in serum analytes but the results are controversial [4]. The analytes studied in the following study to determine the effect of freezing and thawing were total bilirubin and total protein. Bilirubin is a yellow orange pigment formed by the degradation of the red blood cells in the reticuloendithelial system, particularly in spleen and liver. Total serum bilirubin concentration is currently used in the management of neonatal jaundice [5]. Normal level of serum bilirubin is 0.2–0.8mg/dL [6]. Quantitative estimation of serum bilirubin is useful in sub-clinical jaundice, where the demonstration of small increase in serum bilirubin between 1–3 mg/dL is of diagnostic value in clinical jaundice to follow the development and course of the jaundice. High values up to 30 – 40 mg/dL may be reached in babies from mothers who have developed Rh antibodies. In these cases the pigment is bilirubin, which may be deposited in the brain giving marked pigmentation-Kernicterus causing severe damage to the brain and proving fatal in some cases. The serum bilirubin level is an important factor in differential diagnosis of jaundice [7]. Protein takes part in numerous biological functions of body [8]. Normal level of serum total protein is 6-8g/dL [9]. A decrease in the level of total protein may be due to heavy loss of protein into or mal-adsorption of protein from the alimentary tract, decreased formation in the liver, increased catabolism of proteins or insufficient intake of protein in the food. Reduction of serum total protein is one of the causes of edema in liver disease. This may occur in malignant disease of stomach, intestine and pancreas, in peptic ulcer and in deficiency of vitamins especially vitamin D. Loss of protein can occur in a number of diseases of the stomach and intestine and also apparently very occasionally as a primary condition. Increase in total protein is seen in conditions where there is significant increase in dehydration along with chronic infections and other conditions. These include rheumatoid arthritis, tuberculosis [8]. The aim of this study was to know the effect of freezing and thawing on these two biochemical important analytes and study how stability of these analytes changes from time at different temperature.

Materials and methods:

The present study comprised of 20 left over serum samples. Serum samples were collected from the free laboratory of KLE Society's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, Karnataka, India. All the serum samples were analysed by Semi-automated method Estimation of bilirubin was done by Diazo-method of Pearlman and Lee [10] and for total protein by Biuret method. The data obtained was analysed statistically by computing descriptive statistics, mean, standard deviation and percentage difference. The results were considered statistically significant whenever $p \le 0.05$.

Results:

The result of the study was as following – (1) Bilirubin showed decrease values at different intervals of each freezing and thawing cycle. The range of total bilirubin on day 1 was 0.36- 26mg/dL and on day 10 was 0.3- 25.5mg/dL. Mean± Standard deviation on day 1 was 8.72±7.39 and on day 10 was 8.53±7.36 respectively. Percentage change of the range bilirubin from day 1st to 10th day was from 0.31% -18.92% which was found to gradually decrease by 0.04-0.51mg/dl from day 1 to day 10. Mean and standard deviation varied at the percentage change of 4.04% and 4.83% with decrease values of mean by 0.18 mg/dl and standard deviation by 0.17. Graph 1 represents the change in the mean values from day1 to day 10 of total bilrubin.

(2) Protein showed stability upto 10 freeze and thaw cycles. The range of total protein on day 1 was 4.41- 12.34g/dL and on day 10 was 4.15- 12.18g/dL. Mean ±Standard deviation on day 1 was 8.25±2.50 and on

day 10 was 8.22±2.64. Percentage change of the protein values from day1 to day 10 was from 31.25% -5.9% which was found to gradually decrease at the range of 2.75-0.32g/dl from day 1 to day 10. Mean and standard deviation varied at the percentage change of 76% and 8.01% with decrease values of mean by 0.03g/dl and standard deviation by 0.69g/dL. Graph 2 represents the change in the mean values from day1 to day 10 of total protein.

Graph 1: Representing the change in mean values of total bilirubin from day 1 to day 10.
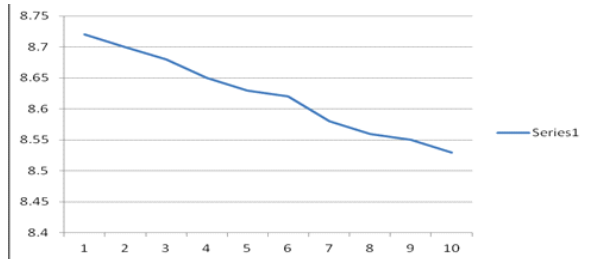
Graph 2: Representing the change in mean values of total protein from day 1 to day 10.
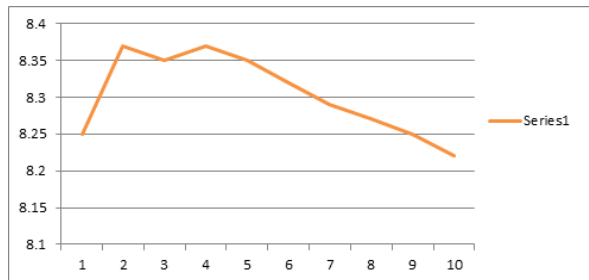
Discussion and Conclusion:

Information on the stability of serum analytes during storage of serum is often incomplete and sometimes contradictory. For clinically useful and reliable results the interval between blood collection and serum separation must be controlled [11]. The two analytes studied under this study are used to study pathological changes in a patient and also monitor different disease. Total bilirubin is photosensitive in nature; there is a known effect of light on the samples for the estimation of total bilirubin many have even concluded that samples for serum bilirubin values should not be placed under the light and if exposed to sunlight to be checked within two hours. Storage affects polypeptides possibly could be due to denaturation occurring by freezing and thawing because of the sensitivity of proteins for the freezing and thawing process this fact is explained when there is low concentration of analytes in serum [12] but many studies have concluded that serum proteins remain stable in storage [13]. The effect of storage and temperature on values of total protein show no or very mild change. Another study concludes total protein stable for 7 days [14]. This study aims at knowing the effect of freezing and thawing on analytes total bilirubin and total protein and also studies how stability of these analytes changes from time at different temperatures. The study also concludes total bilirubin values gradually decrease when we repeatedly freeze at -20°c and thaw at room temperature. Total protein is stable for 10 cycles of freezing and thawing. Hence we need to be cautious while planning the studies and research projects which take up bilirubin as the parameter where repeated freezing and thawing is required.

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