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Clinical Research

STABILITY OF SERUM CALCIUM AND PHOSPHORUS AT DIFFERENT STORAGE TEMPERATURE

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ABSTRACT In the present study, stability of serum calcium and phosphorous was assessed for one year duration of storage at different temperatures (-20°C and -80°C). Forty aliquots each of 200µl were made from pooled serum. Fresh sample was analyzed within 4 hours of collection, considered as baseline value (t0). The serum aliquots were stored at -20˚C & -80˚C respectively which were analyzed over a period of one year and value was compared against baseline. Analysis of serum calcium and phosphorous was done on Automatic Analyzer (Roche Hitachi -902) in NABL accredited laboratory. Data was analyzed for serum calcium and phosphorus values obtained at different time points and storage temperatures (-20°C and -80°C). The results indicate clinically acceptable stability of one year for calcium stored at -20°C with not much gain at -80°C. Phosphorus is stable in serum for one year with better stability at storage temperature -80°C.

KEYWORDS : calcium, phosphorus, stability, storage

INTRODUCTION:

The measurement of biomarkers in blood specimens has become an integral component of many epidemiologic studies. Use of blood biomarkers introduces several decision points at the study design phase for the investigator [1]. Inorganic Phosphorous and Total Calcium are measured to diagnose various disorders including parathyroid gland and kidney disease and Vitamin-D imbalance and bone disease, chronic renal disease etc. A number of factors, primarily preanalytical and analytical or normal biological variations affect the accuracy of test results. Preanalytical factors such sample collection and handling, diet, exercise and drugs can all impact a test result [2].

Collection, processing and purifying of biological materials over and over again is time consuming and labour intensive. Therefore, it is always necessary to keep the biological materials for various time intervals. Storage of the biological materials has been a long-standing challenge for diagnostics, especially those designed for remote places and more numbers of samples. Laboratories usually face problems in analysing samples due to frequent breakdown of equipment and shortage of supply of kits or chemicals which leads to delay in analyzing samples. Besides this, samples are transported to central laboratories from other areas where there is no laboratory facility. Samples which were collected and stored for a long time in various laboratories may be used if the preanalytes are found to be stable. These values may be correlated with other parameters. If the preanalytes are stable, it can be used for the comparison with the current values if so required. If the preanalytes are stable for a long period, the results may be verified with better techniques developed in future. Limited studies available have reported stability of serum calcium and phosphorus for short duration. The present study was carried out to examine the stability of serum calcium and phosphorus stored for a longer duration of one year at two different temperatures (-20˚C and -80°C).

MATERIALS AND METHODS:

Materials: The experiment was conducted from December, 2014 till November, 2015. Informed consent was taken from all volunteers prior to collection of blood. Blood sample was collected from eight health staff of the centre

Blood sampling: Blood collection was made by the phlebotomists through venipuncture. Venous blood (3 millilitre) was drawn and collected in Becton Dickinson (BD) plain vacutainer (3 ml). The vacutainers with samples were allowed to stand at room temperature for 30 minutes to clot and were then centrifuged at 1500 rpm for 10 minutes at room temperature for separation of serum. The serum from all tubes was then pooled.

Sample preparation and analysis: The pooled serum was then divided into 80 aliquots each of 150 µl. Forty aliquots of serum were stored at -20˚C & -80˚C respectively. Two aliquots were run to get duplicate readings. The first batch of aliquots was analyzed within four hours of collection of sample, considered as t0. Each aliquots in duplicate for serum samples which were stored at -20^{0}C and -80^{0}C were analyzed alternate day for one week; weekly for 2 months; twice in a month for 2 months and then after 6 months. Hence, the samples were run for a total of 16 times during the experiment.

Serum calcium and phosphorus was estimated on ROCHE Hitachi Autoanalyzer (902) using diagnostic kits (5-Nitro-5^1-methyl-BAPTA and molybdate UV method respectively) in a National Accreditation Board of Testing and Calibrating Laboratories (NABL) accredited

laboratory of Centre for Promotion of Nutrition Research and Training, with Special Focus on North-East, Tribal and Inaccessible Population (Indian Council of Medical Research), New Delhi.

Statistics: Paired t-test was used for the calculation of the significance of difference between the storage conditions by using Microsoft Excel. Linear regression analysis was used to determine trend of stability with time. Chisquare test and odd ratio was also applied using Microsoft Excel.

RESULTS:

Data was analyzed for serum Calcium (CAL) and Phosphorus (PHOS) values obtained at different time points and storage temperatures (-20°C and -80°C). The Fresh sample values (base values) considered were the mean of the two samples taken initially i.e. at time point t0. The mean values at various time points (t1, t2, t3, t4, t5, t6, t7, t8, t9, t10, t11, t12, t13, t14 and t15) were considered as values of the analytes at different time points.

The stability of analytes at different time points was assessed by relative percentage differences from the base values (Table 1). It was computed at each time point of storage by using the formula, [(Xb - Xt)/Xb]x100, where Xb is the base value (fresh samples) and Xt is its mean value at time points ti (i=1,2,….15).

Table 1. Absolute Relative Percentage Differences in values of analytes over the time points when stored at -20°C & -80°C

Time point	Calcium		Phosphorus	
	Relative % diff at -20°C	Relative % diff at -80°C	Relative % diff at -20°C	Relative% diff at -80°C
t1	1.546392	2	6.323529	1.872075
t2	1.030928	3.5	6.764706	1.404056
t3	1.546392	5	6.029412	1.872075
t4	3.608247	7	5.294118	0.780031
t5	0.515464	6	11.32353	7.020281
t6	0	5.65	5.294118	2.028081
t7	2.57732	0.5	6.029412	4.056162
t8	0.515464	1.5	10.88235	6.708268
t9	4.123711	3.5	9.117647	7.644306
t10	3.092784	2.5	10.73529	6.396256
t11	2.061856	2.5	7.352941	4.524181
t12	2.061856	5	5.294118	2.964119
t13	1.546392	3.5	3.823529	1.24805
t14	3.608247	1	4.558824	6.864275
t15	1.030928	0.5	9.117647	4.836193
Mean	**1.924399**	**3.31**	**7.196078**	**4.014561**
SD	**1.254581**	**2.062956**	**2.44089**	**2.44008**

The stability of the analytes stored at -20°C and -80°C was also studied in terms of distribution of the absolute relative differences in the values of the analytes from the base values to assess the clinical acceptable stability over the storage time points at two specified temperatures. For the purpose, the frequency distributions of absolute relative differences (ignoring positive or negative signs of the values) of the analytes from base values were worked out and are presented in Table 2 for the two storage temperatures.

For the comparison of the stability of the analytes at the two temperatures; we used minimum error criteria, viz. the frequency of Abs % diff less than 4 and computed the 2x2 contingency for the two analytes respectively

Table 2. Distribution of Abs % diff less than 4, for calcium and phosphorus as per the storage temperatures

	CALCIUM		PHOSPHORUS	
	-80°C	-20°C	-80°C	-20°C
<4	10	14	7	1
≥4	5	1	8	14
	X2 = 3.32 (p > .05)		X2 = 6.12(p < .05)	

DISCUSSION:

From Table 1, it is observed that all the values of the relative percentage differences from base values to values obtained at different time points over a period of one year; ranged from 0.0 to 4.123 for the analyte CAL and from 3.823 to 11.323 for the analyte PHOS stored at -20°C. At -80°C, it ranges from 0.5 to 5.65 for CAL and from 0.780 to 7.644 for PHOS. Thus, at storage temperature of -80°C, mean relative percentage difference for both the analytes, CAL (3.31%) and PHOS (4.01%) did not cross the clinical acceptable limit of 10%. However, at -20°C, the percentage differences at all time points remained below the clinical acceptable limit of 10% except for PHOS. At instances, e.g at t5, t8 and t10 it crossed the acceptable limit and at two occasions (t9 and t15) it was near 10%. With respect to PHOS, the minimum limit of % diff was 3.82 at -20° C while it was 0.78 at -80° C. Similarly the maximum limit of % diff was 11.32 at -20° C while it was 7.64 at -80°C favouring the stability of PHOS at -80°C.

Distribution of the absolute relative differences in the values of the analytes from the base values, presented in table 2 provides a picture of the distribution of differences over the period of observations for the two analytes at two temperatures of storage. It is observed that for the analyte CAL, the frequency of lesser % differences (<4%) which is 14, is much more than the frequency of bigger such differences (≥4%) which is 1 in case with temperature -20°C of storage, indicating favour of storage of this analyte at this temperature. A non significant association is noted in terms of X2 test (p>.05) with respect to storage of this analyte at -80°C. Thus little gains are there in favour of storage of this analyte at -80°C compared to its storage at -20°C. However, for PHOS the frequency of lesser % differences (<4%) at -20°C is 1 compared to bigger such differences (≥4%), which is 14. Thus chances of bigger % diff are more in case of this analyte when stored at -20°C. Significant X2 association (p<.05) is revealed for this analyte towards lesser % differences. This supports the contention that stability of the analyte CAL is maintained for the period of observation of one year at storage temperature -20°C. For the analyte PHOS, it shows better stability at temperature -80°C.

Studies have reported stability of serum calcium and phosphorus for short duration. A recent study by Kachhawa et al revealed that serum calcium and phosphorus were stable for 30 days at -20°C [3]. Cuhader et al also revealed that serum calcium was stable for 3 months of storage at -20°C, or up to ten times of freeze-thaw cycle [4]. Stability of calcium has also been reported in rat sera at -20°C for up to 90 days [5].However, our study indicated stability of calcium in serum for longer period (one year) at both -20°C and -80°C.

Similarly for phosphorus, limited studies carried out have reported short term stability in serum. A study carried out among 10 analytes including phosphorus indicate that Glucose, Phosphorus and creatinine were the least stable and the serum should be determined within 48 h at 4±1°C and 24 h at 23±1 °C for these analytes [2]. Dirar et al also reported that serum phosphorus should be determined within 48 hrs at 4±1°C [6]. Heins et al [7] determined 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. However, in our study serum phosphorus were measured within 4 hours and stored at -20°C & -80°C. We observed stability of phosphorus in serum for longer period (one year) at both -20°Cand -80°C.

CONCLUSION:

The results indicate clinically acceptable stability of one year for the analyte calcium stored at -20°C with not much gain with its storage at -80°C. Phosphorus is stable in serum for one year with better stability at storage temperature -80°C.

REFERENCES:

1. Holland NT, Smith MT, Eskenazi B, Bastaki M. Biological sample collection and processing for molecular epidemiological studies. Mutat Res 2003;543: 217–34.
2. Morajini A. Effect of Storage Time and Temperature on Some Serum Analytes. American Journal of Applied Sciences 2008;5(8): 1047-1051
3. Kachhawa K, Kachhawa P, Varma M, Behera R, Agrawal D, Kumar S. Study of the stability of various biochemical analytes in samples stored at different predefined storage conditions at an accredited laboratory of India. J Lab Physicians 2017;9:11-5
4. Cuhadar S, Koseoglu M, Atay A et al. The effect of storage time and freeze-thaw cycles on the stability of serum samples. Biochem Med (Zagreb). 2013;23(1):70-7.
5. Cray, C., Rodriguez, M., Zaias, J. et al. Effect of storage temperature and time on Clinical Biochemical Parameters from Rat Serum. J AM Assoc Lab Anim Sci. 2009; 48 (2): 202-204.
6. Dirar AM, Abdallah DA and Abdelsalam KEA. Effect of Storage Time and Temperature on some Serum Analytes. International Journal of Pathology; 2010; 8(2): 68-71
7. Heins M, Heil W, Withold W. storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes. Eur J clin chem. Clin Biochem 1995; 33: 231-238.