



## STABILITY OF GLUCOSE IN HUMAN SERUM AND PLASMA STORED FOR A LONGER PERIOD OF ONE YEAR AT DIFFERENT TEMPERATURES

### Medical Science

<b>Supriya Dwivedi</b>	Center for Promotion of Nutrition Research and Training with special focus on North-East, Tribal and Inaccessible population (Indian Council of Medical Research), ICMR Campus-II, Tuberculosis Association of India Building (1st floor), 3, Red Cross Road, New Delhi-110001, India
<b>Zaozianlungliu Gonmei</b>	Center for Promotion of Nutrition Research and Training with special focus on North-East, Tribal and Inaccessible population (Indian Council of Medical Research), ICMR Campus-II, Tuberculosis Association of India Building (1st floor), 3, Red Cross Road, New Delhi-110001, India
<b>Ashutosh Kumar Dixit</b>	Desert Medicine Research Centre (Indian Council of Medical Research), New Pali Road, Jodhpur-342005, Rajasthan, India
<b>Priyanka Gupta Bansal</b>	Center for Promotion of Nutrition Research and Training with special focus on North-East, Tribal and Inaccessible population (Indian Council of Medical Research), ICMR Campus-II, Tuberculosis Association of India Building (1st floor), 3, Red Cross Road, New Delhi-110001, India
<b>Suman Sundar Mohanty</b>	Desert Medicine Research Centre (Indian Council of Medical Research), New Pali Road, Jodhpur-342005, Rajasthan, India
<b>Gurudayal Singh Toteja*</b>	Center for Promotion of Nutrition Research and Training with special focus on North-East, Tribal and Inaccessible population (Indian Council of Medical Research), ICMR Campus-II, Tuberculosis Association of India Building (1st floor), 3, Red Cross Road, New Delhi-110001, India *Corresponding Author

### ABSTRACT

Stability of serum and plasma glucose was assessed for one year duration of storage at different temperatures. Eighty aliquots each of 150 µl were made from pooled serum and plasma. Fresh sample was analyzed within 4 hours of collection, considered as baseline value. Aliquots of samples were stored at -20°C & -80°C and were analyzed over a period of one year and values compared against the baseline. All values of the relative percentage differences for serum and plasma glucose, from its values in fresh samples were within acceptable variations of less than 10%. Our study indicates clinically acceptable stability for Plasma Glucose and Serum Glucose over the period of one year of observation, with better stability in case of Serum Glucose at -80°C.

### KEYWORDS

Stability, glucose, serum, plasma

### INTRODUCTION

Stability of analyte is an important pre-analytical factor that may influence the results of any quantitative bioanalytical method. Since biological samples are usually not analyzed directly after collection, but are first transferred to the laboratory, processed and stored, it is essential to ensure that analyte remains stable under storage conditions for a particular duration. This is one of the major concerns especially in large community based studies where samples are collected from far flung places, transported to central laboratories for analysis and are then subsequently analyzed.

Limited studies are available on optimum condition and timeline for the stability of glucose level in the serum and plasma specimen. Hence, we have made an attempt to assess a) stability of glucose upto one year; b) whether there is any difference in stability of serum and plasma samples; c) optimum storage conditions (-20°C and -80°C).

### MATERIALS AND METHODS

Five ml venous blood was drawn from 8 study volunteers after taking informed consent. Blood sample was collected in Becton Dickinson (BD) plain vacutainer (3 ml) and fluoride vacutainer (2 ml) and were allowed to stand at room temperature for 30 minutes to clot. The vials were centrifuged at 1500 rpm for 10 minutes at room temperature for separation of serum and plasma respectively. Serum and plasma was divided into 80 aliquots each of 150 µl. 40 aliquots each of plasma and serum were stored at -20°C & -80°C respectively. Every time when the analysis was done, two aliquots were run to get duplicate readings. The first batch of aliquots was analyzed within four hours of collection of sample, considered as t<sub>0</sub>. Each aliquots in duplicate for both plasma and serum samples which were stored at -20°C and -80°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 and Plasma glucose was estimated on ROCHE Hitachi Autoanalyzer (902) using GOD-PAP method 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.

### RESULTS

The glucose content in Blood Plasma (P) and Blood Serum (S) for the samples stored at -20°C and -80°C was analyzed at different time points. Two aliquots were analyzed at each time point. The base values considered were the mean of the two samples analyzed within 4 hours of collection of sample, considered as t<sub>0</sub>. Two values at each point thereafter for the samples stored at 20°C and -80°C respectively, were taken. The mean values at various time points (t<sub>1</sub>, t<sub>2</sub>, t<sub>3</sub>, t<sub>4</sub>, t<sub>5</sub>, t<sub>6</sub>, t<sub>7</sub>, t<sub>8</sub>, t<sub>9</sub>, t<sub>10</sub>, t<sub>11</sub>, t<sub>12</sub>, t<sub>13</sub>, t<sub>14</sub> and t<sub>15</sub>) were considered as values of the analytes in blood plasma and blood serum.

The stability of analytes at different time points in between was assessed by relative percentage differences from the base values. It was computed at each time point of storage by using the formula,

$[(X_b - X_t)/X_b] \times 100$ , where X<sub>b</sub> is the base value (fresh samples) and X<sub>t</sub> is its mean value at time points t<sub>i</sub> (i=1,2,...15).

The absolute relative percentage differences (ignoring positive or negative sign of the value) of the analytes from their base values, for

storage at -20°C and -80°C are given in Table 1 for different time points under study.

**Table 1: Relative Absolute percentage differences (Abs % diff) in plasma and serum glucose values over the time points when stored at -20°C and -80°C**

Time Points	Sample stored at -20°C		Sample stored at -80°C	
	Plasma glucose	Serum glucose	Plasma glucose	Serum glucose
t1	1.604278	0	0	0.520833
t2	1.604278	1.546392	0	0.520833
t3	2.139037	4.123711	1.630435	2.083333
t4	3.208556	2.57732	0.543478	1.5625
t5	5.347594	5.154639	3.26087	3.645833
t6	0.534759	5.670103	3.804348	2.604167
t7	3.208556	5.670103	1.086957	3.125
t8	3.743316	3.608247	2.173913	3.125
t9	2.673797	4.639175	2.173913	2.604167
t10	5.347594	6.701031	7.065217	5.729167
t11	1.069519	4.123711	2.717391	3.125
t12	1.069519	2.57732	1.630435	3.125
t13	3.743316	1.546392	2.173913	1.041667
t14	5.347594	4.639175	5.434783	4.6875
t15	3.743316	3.092784	0.543478	1.5625
Mean	2.96	3.71	2.282609	2.604167
SD	1.61	1.83	1.915841	1.410423

The stability of the analytes stored at -20°C and -80°C was also studied in terms of the distribution of the absolute relative differences in the values of the analytes from the base values over the period of storage. For the purpose, the frequency distributions of absolute relative differences of the analytes from base values were worked out. The frequency of Absolute percentage difference (Abs % diff) less than 4 or more, for the comparison of their stability at the two temperatures is given in Table 2.

**Table 2: Distribution of Abs % diff with a cut of point (viz.4), for Plasma and Serum as per the storage temperatures**

Abs % Diff	Plasma Glucose		Serum Glucose	
	-80°C	-20°C	-80°C	-20°C
< 4%	13	12	14	7
≥ 4 %	2	3	1	8
	$\chi^2=0.24(p>.05)$ OR= 1.6		$\chi^2=10.30(p<.05)$ , OR=16	

## DISCUSSION

Glucose level is measured in both plasma and serum samples. A study indicated that serum gave values lower than fluoride plasma by 1.15% (Frank *et al* 2012). While Ladenson *et al* (1974) and Breier *et al* (2014) reported lower glucose concentration in plasma compared to serum. Our study has been attempted to analyse the storage of Plasma glucose and Serum glucose for a longer period (one year) at two temperatures (-20°C and -80°C).

From table 1, it is observed that all the values of the relative percentage differences, which happen to be the relative differences in the values of the Plasma Glucose and Serum Glucose from its values in fresh samples expressed in percentage, taken at different time points over a period of one year; were also within 2 SD limits, indicating acceptable variations over the period of time, the analytes were kept at these temperatures. These differences are less than the clinically accepted limit (less than 10% relative differences) (Heins *et al*1995). The absolute % differences ranged from 0.53% to 5.54% for Plasma and from 0.0% to 6.70% for serum when kept at -20°C. In case of keeping the samples at -80°C, these ranges have been, 0-7.06% in case of plasma and from 0.52-5.70% in case of the serum. Thus nowhere the % differences crossed the clinical acceptable limit of 10%. These observations support the contention that stability of the two analytes are maintained within in clinical acceptable limits over a storage period of one year at both temperatures of storage. The difference between the mean values of the relative % differences for the plasma at temperature -20°C and -80°C was found statistically non significant ( $t=1.096$ ,  $p>.05$ ) but the difference between the mean values of the relative % differences for the serum at these temperatures was towards significant ( $t=1.88$ ,  $p<.05$ ), which indicates that statistically, on an average the percentage differences at both the temperatures of storage were

comparable in case of Plasma but not so in case of Serum. Hence, glucose is found stable in plasma whether stored at either -20°C or -80°C for a period of one year. In case of serum glucose stability, we have different views in the literature. A recent study in Korea to assess stability of 17 routine chemistry analytes like glucose, albumin, HDL, uric acid, sodium etc at 22°C, 4°C and -66°C for 30 days, reported serum glucose to be stable at all three temperatures (An and Park, 2014). Cuhadar *et al* (2013) reported that serum glucose concentration was stable for three months when stored at -20°C, or up to ten times of freeze-thaw cycle. A study to assess effect of storage temperature and time on clinical biochemical parameters from rat serum indicated 5.8% and 1.3% loss from the baseline value of serum glucose when stored at -20°C and -70°C respectively for 360 days (Cray *et al*2009). Miles *et al* (2004) reported that serum and heparin plasma values stored at -70°C for up to 8 months showed comparable values for glucose concentrations. Another study reported serum glucose to be stable at both 4°C, and -20°C for 4 months (Donnelly *et al*1995).

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 from this table that higher % differences from the base (more than 4%), in case of plasma glucose are comparable at the two temperatures (frequency of differences 3 and 2 respectively), but a high reduction in case of serum glucose (frequency of differences 8 at temperature -80°C, which is reduced to 1 at temperature -20°C). The differences are heavily reduced when the serum is stored at -80°C. This observation is supported by significant  $\chi^2$  value ( $p<.05$ ) and OR=16, in case of comparison of storage of serum at the two temperatures.

The results thus indicate clinically acceptable stability for plasma glucose and serum glucose for storage duration of one year at -20°C and -80°C. Plasma glucose showed comparable stability at both -20°C and -80°C, while serum glucose showed better stability at -80°C. More studies in this regard are required.

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