International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Effect of Storage on the Stability of Enzyme Activities in Pooled Serum

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ABSTRACT

Objective: To assess the stability of enzymatic parameters in home-made Frozen Human Serum on storage and compare it with commercially prepared lyophilized human sera already being used in our laboratory at IGIMS, Patna.
Methods: The home made QC serum was prepared from twenty healthy volunteers and was screened for HIV and HBV, pooled together and stabilized with 0.1% Sodium azide. Preliminary control limits (i.e. Mean, SD) was calculated from 20 runs of first month for three enzymatic parameters and results were compared with those of commercially available lyophilized human sera.
Results: ALP had narrower coefficients of variation in the home made serum making it a stable control material in comparison to the commercial ones whereas other enzymatic parameters showed unsteadiness over the 6 months storage period.
Conclusion: We conclude that the activity of ALP in pooled sera was stable when stored frozen at -20^{0}c over the 6 months storage period.

Key Words: *enzymatic parameters, lyophilized human sera, pooled, storage.*

INTRODUCTION

QC techniques are used for day-to-day monitoring of the reliability of Clinical Biochemistry laboratory performance. Application of these techniques will help reduce errors and give both the laboratory and the clinician confidence in the results. Laboratories can be assessed on their three phases of functioning, Pre-analytical, analytical and post analytical phases. [1] While pre-analytical and postanalytical errors are more managerial issues, Internal Quality Control (IQC) and External Quality Assessment (EQA) schemes can monitor the analytical variability. [2] External quality control helps in comparing our results with other laboratories and to know the systemic errors i.e. poor accuracy. Internal quality control helps in minimizing the random errors i.e. poor precision. [3]

Through the quality control (QC), these errors can be minimized and the laboratory can ensure about the reliability of its results. Incorrect laboratory results may lead to the wrong management and possible fatal results. [4] The reliability of laboratory results is therefore most important. However, many developing countries are disadvantaged by unavailability and high cost of commercial QC materials. [5] Therefore preparation of Home-made pooled human serum will be a very cost effective way for use as QC material. Control material prepared should simulate fresh human serum in terms of matrix and concentration of analytes. [6] It must be

sufficiently homogenous, stable and non-infective. There are two main sources of QC materials, animal and human. [7] The animal serum is disadvantageous due to matrix effect which requires supplementation with enzymes and other constituents. [8] Therefore, human serum is suitable for preparing control materials in clinical chemistry. Three types of QC materials can be prepared, frozen serum, stabilized liquid serum and Lyophilized or freeze dried serum. [9,10] Frozen stabilize sera is more consistent than lyophilized serum as there is always reconstitution error which can vary results from vial to vial. So, we intended to study the stability of home-made pooled sera human serum with stabilizers and check the durability of the home-made QC material, estimated the loss of activities at the end of the study and compared the results with commercial QC control material kept at the same condition.

MATERIALS AND METHODS

This study was a prospective analytical study, performed in the Clinical Biochemistry Laboratory of Indira Gandhi Institute of Medical Sciences, Patna. IQC tests were performed based on biochemical tests from the pooled sera, run for 6 months from 7th April 2017 to 8thOctober 2017. Serum was obtained from 20 healthy volunteers in IGIMS, Patna. Samples were tested for antibodies against HIV and HbsAg. Serum from each donor was pooled to obtain total volume of about 450 ml in a graduated conical flask. Pooled serum was processed, adjusted the levels and placed in a deep freezer (-15 to -20^{o}C) to completely freeze the pooled serum. Next day, the conical flask containing the frozen-pooled serum was kept at room temperature and it was allowed to completely thaw. 0.1%Sodium Azide as preservative agent was added. The serum was then mixed thoroughly with Sodium Azide and aliquots of 0.5 ml were made .Aliquots were stored in a deep freezer (-15 to -20^{o}C) until analyzed.

Comparative study was done between homemade pooled sera and commercial QC material stored at – 20^{o}C. Daily one aliquot of pooled was subjected to analysis for twenty days in a month. At the end of six months means of the results were compared to the initial value .(Preliminary control limits (i.e. Mean ,SD) was calculated from 20 runs of first month).Two levels of commercial control material were reconstituted each month and were run along with pooled sera. We used Excel analysis pack to test for equality of variance by Levene's test and t test for equality of the means at 95 % confidence interval.

The means of the analytes from pooled sera and commercial control materials showing the p values < 0.05 (Table 1, 2, 3) at -20^{o}C were plotted on the linear graph. The purpose was to examine for the deviation and differences from initial values and results from the sample stored at -20^{o}C. The percentage change in concentration of each analyte after six months of storage period was computed for all the three materials. The Frozen serum stabilized with Sodium Azide was introduced as a new lot of normal control on OLYMPUS AU400 fully automated analyzer along with the commercial lyophilized human control sera -Level-1 (Normal)(Biorad) and Level-II (abnormal or higher)(Biorad), for comparison of the following three enzymatic parameters being analyzed routinely on the instrument.

- Alanine Aminotransferase (ALT)
- Aspartate Aminotransferase (AST)
- Alkaline Phosphatase

RESULTS

At 95 % confidence interval the results of the pooled sera sample stored at -20^{o}C were statistically compared with results of initial values. The analytes were considered stable, if the values did not differ significantly (P >0.05).Significant p values < 0.05 were seen in SGPT, for pooled sera sample (Table 1). Analyte showing p value < 0.05 in commercial control material level 1 were ALP, and SGOT.(Table 2). There

was no significant changes seen with commercial QC control material level 2(Table 3**).**

Table 1: Comparison between initial value and end of six month (pooled sera stored at -20°C)

ANALYTES	UNIT	*n*	INITIAL		AT 6TH MONTH END		p val (0.05)
			MEAN	SD	MEAN	SD	
SGOT	IU/L	20	41.94	3.86	34.72	0.40	0.503
SGPT	IU/L	20	28.51	1.00	26.89	0.79	<0.001
ALP	IU/L	20	101.91	10.64	103.28	10.01	0.685

Table 2: Comparison between initial value and end of six month (commercial QC level 1stored at -20°C)

ANALYTES	UNIT	*n*	INITIAL		AT 6TH MONTH END		p val (0.05)
			MEAN	SD	MEAN	SD	
SGOT	IU/L	20	48.2100	1.5614	49.7950	1.6998	0.0048
SGPT	IU/L	20	46.0600	9.6598	46.5700	1.3499	0.8220
ALP	IU/L	20	147.1300	20.0165	131.7000	4.0632	0.0035

Table 3: Comparison between initial value and end of six month (commercial QC level 2 stored at -20°C)

ANALYTES	UNIT	*n*	INITIAL		AT 6TH MONTH END		p val
			MEAN	SD	MEAN	SD	
SGOT	IU/L	20	131.1	9.038252	131.5	5.37122	0.869363
SGPT	IU/L	20	127.95	8.393301	127.95	8.634089	1.00
ALP	IU/L	20	539.85	95.11	485.05	84.66	0.068575

The percentage changes in concentration of analytes in pooled sera sample, commercial QC control material level 1 and 2 stored at –20°Cfor 6 months were compared against initial values (Table 4). At –20°C, the maximum changes were shown by SGOT & SGPT (decreased by 17 % and 6% respectively) at the end of 6 months for pooled sera.In Commercial control material level 1 maximum percentage changes were observed in ALP (decreased by 11%) and SGOT (increased by 3.3%) .

Table 4 : Percentage changes in concentration of analytes at the end of 6 months

ANALYTES	Pooled sera	Level 1	Level 2
AST	-17.2	3.3	0.3
ALT	-5.7	1.1	0.0
ALP	1.3	-10.5	-10.2

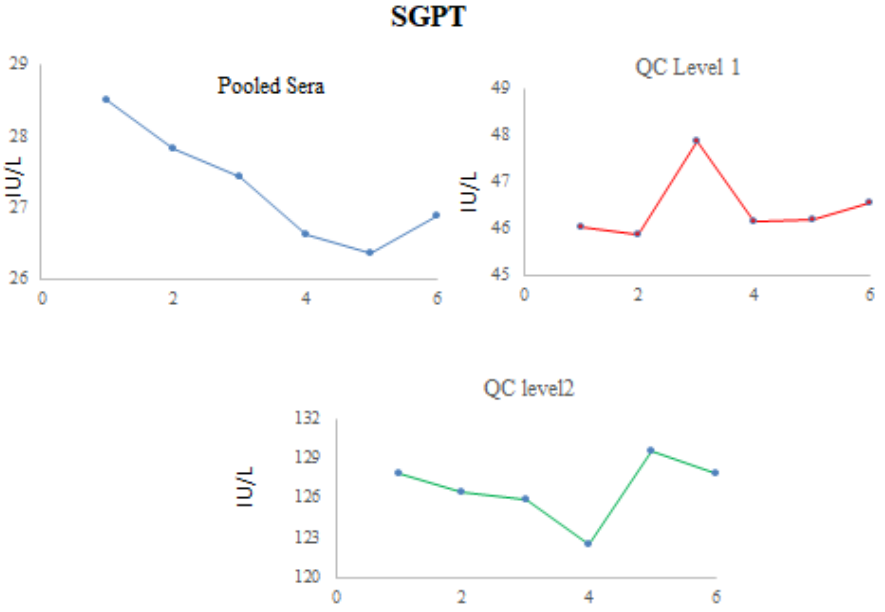

Figure1: Linear graph of SGPT in pooled sera & commercial controls.(displays unsteadiness throughout the study period in both pooled sera and commercial controls)

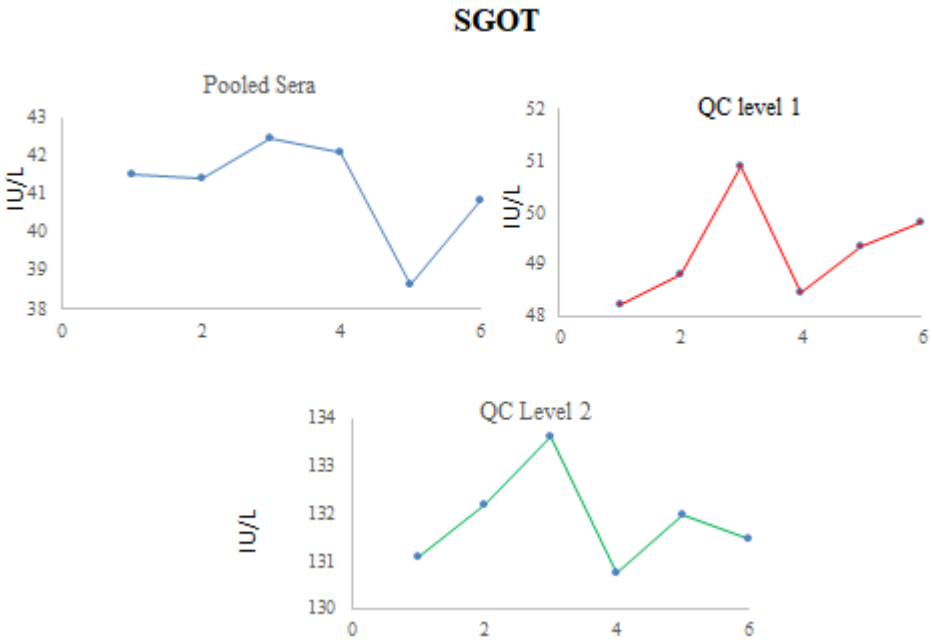

Figure2 :Linear graph of SGOT in pooled sera& commercial controls(shows marked variations in pooled sera as well as commercial controls)

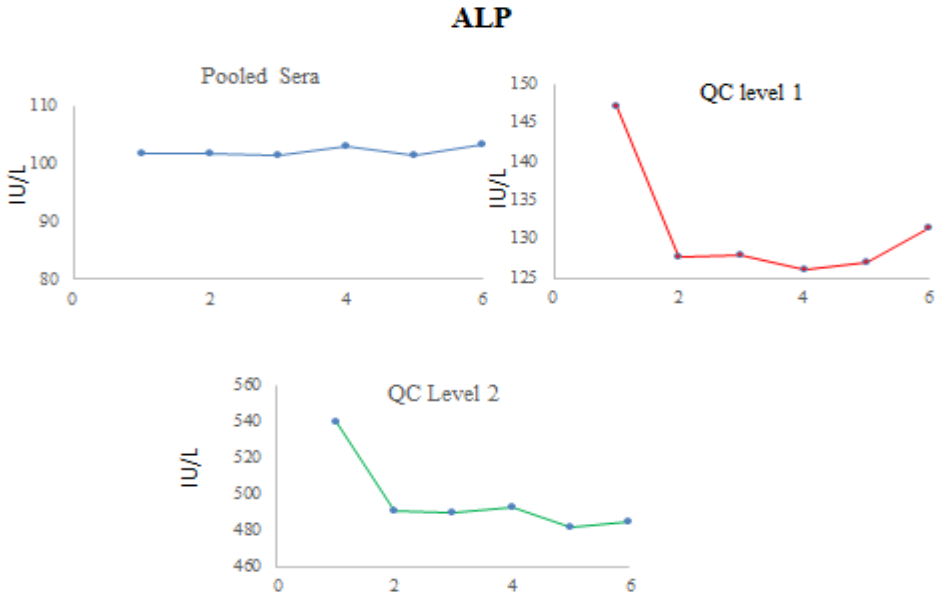

Figure3: Linear graph of ALP in pooled sera & commercial controls.(nearly constant values of ALP in pooled sera displays stability throughout the study period as depicted in straight line)

Table 5: Comparision of % CV in the home made serum vs the commercial sera.

Analytes	Pooled Sera CV%	QC level 1 CV%	QC level 2 CV%
ALT	2.7	1.5	1.8
AST	3.0	1.8	0.7
ALP	0.7	5.5	3.9

The narrower coefficients of variation in the home made serum versus the commercial sera imply a lesser vial to vial variation of the constituent analytes in the home made serum translating into better potential for error detection in the normal ranges. Alkaline Phosphatase has less %CV in the home made serum (as shown in green box) and this leads to the long term stability

whereas ALT and AST had marked variation as depicted in Table 5.

DISCUSSION AND CONCLUSION

With increasing automation in the laboratory, the requirements for quality control material have greatly increased in order to monitor performance. The constant use of commercial control is not economically feasible for many other developing countries because of the non-availability and/or the high cost of these materials. [11-13]

We carried out stability tests for the pooled sera sample and commercial control material stored at –20^{o}C. The results were statistically compared with the initial values for equality of variance using Levene's test and equality of mean using t test at 95% confidence interval. There are many literatures describing the instability of the biological compounds in lyophilized and liquid serum stored at various temperatures. [14-16] Decrease in SGPT and SGOT level (depicted in the Fig 1&2) in our pooled sera samples could be due to the loss of enzyme activities in prolonged storage and the interference by LDH on SGPT should also be considered. Linear graph of ALP in pooled sera shows stability throughout the study period.

The variety of statistical approaches used to assess stability also affects the stability results. It has been reported that about 5 % of the results are expected to show instability due the choice of the statistics. Therefore, recommended alternate approaches to using statistics in order to provide useful information depending on the nature and frequency of the measurement. ALP had narrower coefficients of variation in the home made serum making it a stable control material in comparison to the commercial ones. The marked variation of ALT and AST in commercial control materials showed that our pooled sera samples can be used as stable control material for ALP in comparison to the commercial ones.

Funding: Self
Conflict of Interest: None declared
Ethical approval: Taken

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How to cite this article: Prasad P, Kumar R, Kumar S. Effect of storage on the stability of enzyme activities in pooled serum. International Journal of Research and Review. 2019; 6(7):121-126.

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