

**Research Article** 

ISSN: 2455-8990 CODEN(USA): CRJHA5

# **Comparative Analysis of Effects on The Clinical Level of Metabolic Parameters After Freeze Thaw Cycles at Various Time Intervals**

# Junaid Mahmood Alam<sup>1</sup>, Zeeshan Ahmed<sup>2</sup>

<sup>1</sup>Professor, Head and Chief Scientist

<sup>2</sup>Associate Scientist and Technical Supervisor

Department of Biochemistry lab services and Chemical Pathology, Liaquat National Hospital and Medical College, Karachi. Pakistan.

Corresponding author's Email: dr\_jmalam@hotmail.com

#### Abstract

The optimal temperature usually used for storage at room temperature is 23°C to 25°C and for storage in freezer is - 2°C at average. For Storage in Refrigerator for the samples that needs to be stored for longer period of time the proposed temperature recognized by international organizations is 2 to 8°C. Any deviation within the afore mentioned temperatures and storage conditions results in deleterious effects and outcomes which can hinder or misjudged by clinicians, attending physician and laboratory analysis. The present study described comparative regression analyses of three metabolic components such as BSR (Blood Sugar Random) Cholesterol and triglycerides to determine effects of freeze Thaw cycle and storage period and temperature to suggest rectification controls and precision methodologies to sustain laboratory services. Data analysis manifested that BSR is the only metabolic component that get affected severely with deteriorating percentage of 44.6% at 24hrs and 20.24% at 48 hrs. Furthermore, triglyceride, got moderately affected whereas cholesterol doesn't get affected at all.

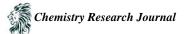
Keywords: Metabolic Parameters, Blood Sugar Level

## 1. Introduction

Clinical laboratories working based on various steps of phases, such as Pre, intra/analytical and post-analytical phases, of which pre-analytical phase is of highest significance. It is well reported that per-analytical phase of errors incurred in it, is the most critical phase or process as part of total analytical process and has immense effect on clinical chemistry testing, reports, clinical decisions and patient wellbeing [1].

Routinely patient samples received from collection points and transferred to analytical sections of laboratories as per specialty. It get analyzed either immediately or stored to complete the batch. In this scenario, storage temperature or if samples were freeze (plasma or serum), its thaw or if and when required, repetition of analysis to further confirm the reported results, becomes very significant. In larger setup laboratories, tertiary care hospital-based labs, samples-testing confirmations, retesting, re-confirmations are standard practice and ensured quality assured services, which are both sustainable and profitable for the patients and patterns [2].

Optimal storage Temperature for samples received either from within the laboratory or OSR or collection point out stationed shall be from internationally recognized protocols procedures and SOPs. The optimal temperature usually



used for storage at room temperature is 23°C to 25°C and for storage in freezer is -2°C at average. For Storage in Refrigerator for the samples that needs to be stored for longer period of time the proposed temperature recognized by international organizations is 2 to 8°C. Any deviation within the afore mentioned temperatures and storage conditions results in deleterious effects and outcomes which can hinder or misjudged by clinicians, attending physician and laboratory analysis. There are a number of articles published in last one decade which reported reviewed analyzed and proposed deviations, errors untoward effects and rectifications regarding abnormalities [1,2]. Long term storage and freeze/Thaw cycles of stored plasma or serum samples has been reported to effect the quality of sample and analytical levels which certainly causes untoward effect clinician decision's patients treatment management diagnosis and sometimes prognosis as well. To rectify, resolved, controlled. Manage and review such abnormal services all clinical laborites needs to process such samples using precision accuracy and the regression co relation analysis to judge assess evaluation and standardized system quality checks and sustainability of services [3]. The present study described comparative regression analyses of three metabolic components such as BSR (Blood Sugar Random) Cholesterol and triglycerides to determine effects of freeze Thaw cycle and storage period and temperature to suggest rectification controls and precision methodologies to sustain laboratory services.

#### 2. Material and Methods

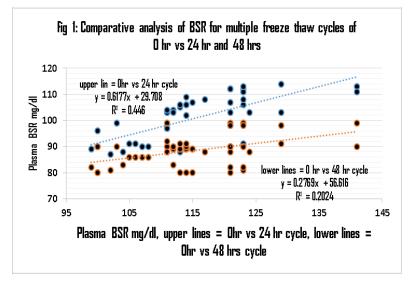
To assess analyses of three metabolic components such as BSR (Blood Sugar Random) Cholesterol and triglycerides to determine effects of freeze Thaw cycle and storage period and temperature to suggest rectification controls and precision methodologies to sustain laboratory services. Thirty-five blood samples, plasma removed, were used and two different temperatures for two different storage periods i.e twenty-four hours and forty-eight hours. Data were analyzed using regression correlation calculation and Y intercept Plotting and comparing the concentration at zero, 24 hrs. Vs 48 hrs. to determine the extent of deviations and the analytical outcomes.

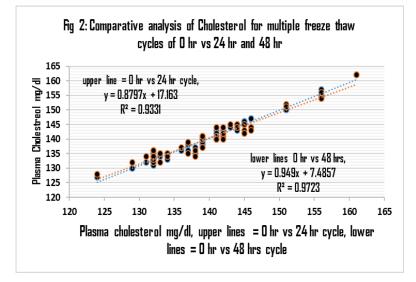
#### 3. Results

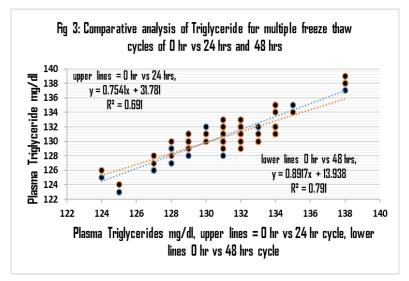
Results are summarized in figures 1 to 3 for BSR, Cholesterol and triglyceride respectively. Figure 1 manifested Comparative analysis of BSR for multiple freeze thaw cycles of 0 hr. Vs 24 hr. and 48 hr. Moreover, comparison for 0 hr. Vs 24 hr. Cycle, y = 0.6177x + 29.708,  $R^{1} = 0.446$ , Lower lines = 0 hr Vs 48 hr. Cycle, y = 0.2769x + 56.616.  $R^{1} = 0.2024$ . The difference between 0 to 24hr has been detected as 44.6% whereas that of 0 to 48hrs as 20.24%. The data clearly assured deterioration in the concentration level of BSR during 48 hr. (Figure 1).

Comparative analysis of Cholesterol for multiple freeze thaw cycles of 0hr Vs 24hrs and 48hr. Upper lines = 0 hr. Vs 24 hr. Cycle, y = 0.8797x + 17.163,  $R^2 = 0.9331$ , Lower lines = 0hr Vs 48 hr. Cycle, y = 0.949x + 7.4857,  $R^2 = 0.9723$ . The difference between 0 to 24hr has been detected as 93.31% whereas that of 0 to 48hrs as 97.23%. In this case freeze thaw cycle even at 48 hr. doesn't affect the integrity of the samples depicted clearly by the higher percentage of regression correlation outcomes (Figure 2). Comparative analysis of Triglyceride for multiple freeze thaw cycles of 0hr Vs 24hrs and 48hr. Upper lines = 0 hr. Vs 24 hr. Cycle, y = 0.7541x + 31.781,  $R^3 = 0.691$ , Lower lines = 0hr Vs 48 hr. Cycle, y = 0.8917x + 13.938,  $R^3 = 0.791$ . The difference between 0 to 24hr has been detected as 69% whereas that of 0 to 48hrs as 79.1%. In case of triglyceride there expected deterioration is a bit moderator manifested both at 24 hr. and 48 hr (Figure 3).











#### 4. Discussion

The present study assessed analyses of three metabolic components such as BSR (Blood Sugar Random) Cholesterol and triglycerides to determine effects of freeze Thaw cycle and storage period and temperature to suggest rectification controls and precision methodologies to sustain laboratory services. Thirty five blood samples, plasma removed, were used and two different temperature's for two different storage periods IE twenty four hours and forty eight hours. Data were analyzed using regression correlation calculation and Y intercept Plotting and comparing the concentration twenty fours hrs. Vs forty eight hrs. to determine the extent of deviations and the analytical outcomes [1-3].

In last decade several dozen of studies regarding Pre analytical phase reported describing a number of errors deviation anomalies for a number of analytical components which are directly related to patient's metabolic status clinical activities physiology and wellbeing as per assessment judgement recommendations and diagnosis of attending physicians clinical aboratory analyst and technologist is the deleterious effects caused by repeated free Thaw cycles of stored samples note and comment needed to be add.

Enzymes are one of most sensitive analytical components that gets effected by freeze Thaw cycle. However there are certain non-enzymatic organic metabolic components that usually get effected such as BSR Cholesterol Triglyceride Urea and bilirubin over present study corroborated the earlier studies reported in journals and periodic of international standardization quality control and clinical chemistry laboratory services. Moreover it is necessarily not only to control the pre analytical errors such as analyzed in present study and reported earlier but the personnel analyst technologist and even senior technical supervisor's needed to be trained upgraded and provided with newer knowledge. To provide sustainable feasible quality assured standardize precise and accurate clinical laboratory services to the end users clinician's customers and patterns [1-3]

#### 5. Conclusion

The present study described comparative regression analyses of three metabolic components such as BSR (Blood Sugar Random) Cholesterol and triglycerides to determine effects of freeze Thaw cycle and storage period and temperature to suggest rectification controls and precision methodologies to sustain laboratory services. Data analysis manifested that BSR is the only metabolic component that get affected severely with deteriorating percentage of 44.6% at 24hrs and 20.24% at 48 hrs. Furthermore, triglyceride, got moderately affected whereas cholesterol doesn't get affected at all.

## References

- [1]. Chuhadar S, Koseoglu M, Atay A, Diricam A. The effect of storage time and freeze-thaw cycles on the stability of serum samples Sera Biochemia Medica 2013;23 (1):70–7 http://dx.doi.org/10.11613/BM.2013.009
- [2]. Rachel C. Williams, William B. Perry, Kathryn Lambert-Slosarska, Ben Futcher, Cameron Pellett, India Richardson-O'Neill, Steve Paterson, Jasmine M.S. Grimsley, Matthew J. Wade, Andrew J. Weightman, Kata Farkas, Davey L. Jones. 2024 Examining the stability of viral RNA and DNA in wastewater: Effects of storage time, temperature, and freeze-thaw cycles, Water Research, 259 (2024), 121879, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2024.121879.
- [3]. Rhim H, Kwag C, Han JI. 2024. A preliminary study on effects of storage time and repeated freezingthawing on the stability of avian serum amyloid A. Acta Vet Scand 66, 42 https://doi.org/10.1186/s13028-024-00764-8
- [4]. Castejon MJ, Rosemeire Yamashiro, Camila C. Oliveira, Elaine L. Oliveira, Edilene P. R. Silveira, Carmem Aparecida F. Oliveira. 2017. Effect of multiple freeze-thaw cycles on the stability of positive antitreponemal serum samples. LABORATORY MEDICINE, J. Bras. Patol. Med. Lab. 53 (4). https://doi.org/10.5935/1676-2444.20170038.

