Pharmacology & Pharmacy, 2023, 14, 478-481
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Storage of Gamma-Glutamyltransferase from Dried Serum Spots: Matrix for Field Based Studies

Ram Kumar, Rizwana Quraishi*

National Drug Dependence Treatment Center, All India Institute of Medical Sciences, New Delhi, India
Email: kram952@gmail.com, *rizwanaquraishi@gmail.com

How to cite this paper: Kumar, R. and Quraishi, R. (2023) Storage of Gamma-Glutamyltransferase from Dried Serum Spots: Matrix for Field Based Studies. *Pharmacology & Pharmacy*, **14**, 478-481.
https://doi.org/10.4236/pp.2023.1411031

Received: October 19, 2023
Accepted: November 25, 2023
Published: November 28, 2023

Copyright © 2023 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

Background: Gamma-glutamyltransferase is recognised as a biomarker to assess the harms associated with alcohol misuse. The objective ways to measure GGT in areas lacking central lab facilities are desirable. This study aims to measure GGT from dried serum spots and its storage from dried serum spots. **Method:** The study was approved by the institutional ethical committee. One hundred and eighty (180) patients were included in the study. Their blood samples were collected. The serum samples were spotted onto filter paper (Whatman 903) dried and stored at 4˚C. The GGT levels were measured on the day of collection and at various time periods to assess the effect of storage. All the analysis was performed on SPSS version 21. **Result:** The GGT levels measured from fresh serum GGT levels mean (SD) 286.5 (539.4) correlated well with their respective dried serum levels 287.18 (538.2) (P = 0.80). The mean recovery of GGT from dried serum was observed to be 103.3%. A sub-sample (n = 12) was stored at 4˚C. The dried serum spots were found to be stable at the end of four weeks using repeated measure analysis of variance (ANOVA) (P = 0.39). **Conclusion:** This method has the potential to be used for epidemiological or field based studies to assess harms associated with alcohol use.

Keywords

Storage, Gamma-Glutamyltransferase, Dried Serum Spots

1. Background

Harmful alcohol use causes more than 5% of the global burden of disease [1].

Identification of harmful alcohol use may help to plan an early intervention. The objective way to assess the damage caused by problematic alcohol use involves laboratory testing. Serum Gamma-glutamyltransferase (GGT) testing is widely used as an alcohol misuse biomarker. Recently serum GGT is established as a superior marker for future disease risk in various clinical setting including epidemiological studies [2]. Epidemiological studies often require field sampling and an alternate method for sample collection and transportation. The use of filter paper for sample collection and storage has many advantages including ease of collection and transportation [3]. It has been reported earlier to efficiently measure various biochemical analytes like aminotransferases, urea and creatinine [4]. Earlier we reported adaptation of GGT assay on filter paper for screening of harmful alcohol use in community setting [5]. The aim of this study is to observe the stability of the GGT assay on filter paper for various time points.

2. Methods

The study was carried out at a leading tertiary care centre for substance abuse treatment. A large number of subjects visit the centre for their problematic alcohol use. The study was performed as per the 1984 Helsinki declaration. The study protocol was approved by the ethical committee at All India Institute of Medical Sciences (AIIMS), Delhi in the year 2015. Clinical validation of the GGT filter paper assay was carried out in one hundred and eighty (180) patients who visited the laboratory for biochemical investigations. After collection of the blood sample the serum was separated. On the day of collection GGT was measured from serum samples as per the standard assay using chemistry analyser from Beckman Coulter. The remaining samples were spotted (20 uL per spot) onto filter paper (Whatman 903). Serum based calibrator and controls levels 1 and 2 for GGT were simultaneously spotted onto filter paper and treated the same way. The filter paper were dried at room temperature (24˚C - 27˚C) for two hours and stored at 4˚C. Next day the spots were punched using a manual puncher. Four spots (6 mm) were placed into a microtube. Extraction was carried out in 250 µL of buffer reagent as described earlier [5]. Comparison of GGT levels obtained from serum and dried serum spots was done using paired T test. The effect of storage on filter disc was assessed at the end of one week, two week and four weeks using repeated measure analysis of variance (ANOVA). All the analysis was performed on SPSS version 21.

3. Result & Discussion

The GGT levels of the fresh serum samples varied between 9 U/L and 3882 U/L (n = 180). The fresh serum GGT levels mean (SD) 286.5 (539.4) correlated well with their respective dried serum levels 287.18 (538.2) using Paired sample T test (P = 0.80; df 179) 95% CI (−2.12 to 1.58). The assay was found to be linear upto 1200 U/L. Ten samples with GGT levels above the linearity limit also correlate well with their filter paper levels. Intra class correlation was calculated to esti-

mate the limits of agreement. Regression analysis reported an R value of 0.99 and an intra-class correlation (ICC) value of 0.99 (Figure 1). The mean recovery of enzyme from dried serum was 103.3% on the day of collection. The mean intra and inter assay coefficient of variance for GGT in dried serum samples were 4.02% and 5.15% respectively.

To study the effect of storage, sub-sample of dried serum spots including quality controls (n = 12) were stored at 4˚C. At the end of one week, two week and four weeks the disc were analysed in duplicates to assess GGT stability. The dried serum spots were reported to be stable at the end of four weeks using repeated measure analysis of variance (ANOVA) (df 4, F1.049, P = 0.39) (Table 1).

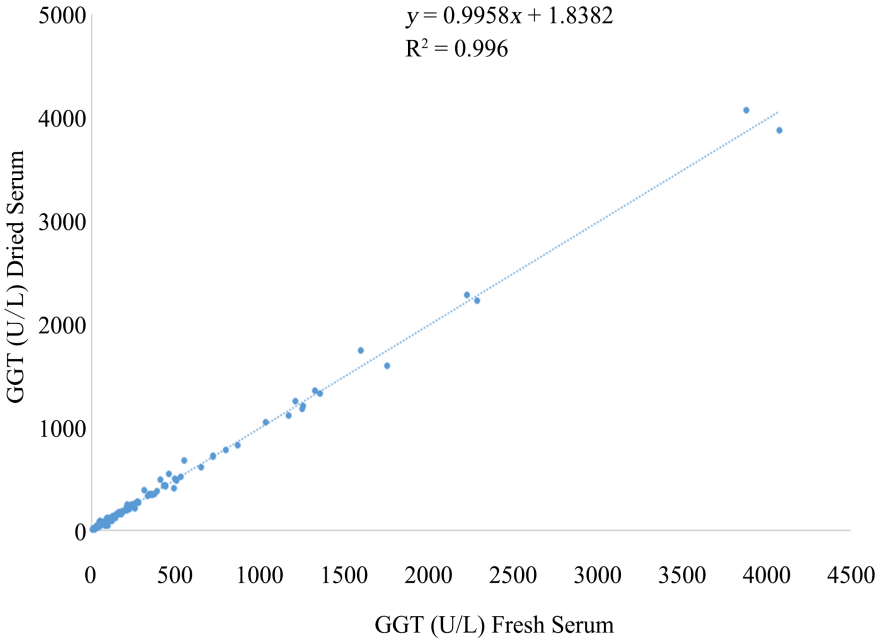

Figure 1. Measurement of GGT from dried serum spots and corresponding serum samples on the day of collection (n = 108).

Table 1. Effect of storage of dried serum spots on GGT levels.

Days of storage (dried serum)	Serum GGT Levels (U/L) Mean (SD) (N = 12)*
Day 0 Direct	346.2 (436.16)
Day 0 dried serum	339.35 (440.53)
Week 1	322.13 (409.08)
Week 2	328.22 (402.12)
Week 3	325.07 (406.48)
Week 4	312.67 (393.19)

*All the samples run in duplicate and compared using repeated measure analysis of variance (ANOVA) (df 4, F = 1.049, P = 0.39).

This report presented the clinical utilization of GGT measurement from dried serum spots in a clinical setting. It is noteworthy to mention the limitation of the study. The filter paper assay may sometimes involve human errors during spotting and punching. Proper drying and storage under desiccants is a prerequisite for filter paper assay. In spite of above limitations the developed assay can be used in clinical assessment of patients with alcohol use disorder with varying GGT levels. Dried serum matrix used in the study stabilises the enzyme for up to four weeks at 4˚C. Thus present method may be adoptable for epidemiological or field based studies and is well comparable to the conventional method.

Financial Support and Sponsorship

The study is funded as intramural research by the All India Institute of Medical Sciences, New Delhi, India.

Conflicts of Interest

There are no conflicts of interest.

References

[1] Poznyak, P. and Rekve, D. (2018) Global Status Report on Alcohol and Health 2018. World Health Organization, Geneva.

[2] Koenig, G. and Seneff, S. (2015) Gamma-Glutamyltransferase: A Predictive Biomarker of Cellular Antioxidant Inadequacy and Disease Risk. *Disease Markers*, **2015**, Article ID: 818570. https://doi.org/10.1155/2015/818570

[3] Mei, J.V., Alender, J.R., Adam, B.W. and Hannon, W.H. (2001) Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens. *The Journal of Nutrition*, **131**, 1631S-1636S. https://doi.org/10.1093/jn/131.5.1631S

[4] Quraishi, R., Lakshmy, R., Mukhopadhyay, A.K. and Jailkhani, B.L. (2014) Transaminase Extraction and Stability from Dried Serum Samples. *Scholars Journal of Applied Medical Sciences*, **2**, 169-170.

[5] Quraishi, R., Varshney, M., Singh, A., Singh, D., Kumar, M., Rao, R., Jain, R. and Ambekar, A. (2019) Use of Filter Paper to Measure Alcohol Biomarkers among Opioid-Dependent Patients on Agonist Maintenance Treatment: A Community-Based Study. *Indian Journal of Psychological Medicine*, **41**, 529-534. https://doi.org/10.4103/IJPSYM.IJPSYM_304_19