International Journal of Medical Toxicology & Legal Medicine **Volume 28, No. 1, 2025**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V28I5/024

Utility of Dried Serum Spots for Sample Storage and Estimation of Cholesterol Levels

Dr Reshma D Channashetti

Assistant Professor, Department of Biochemistry, KLE Academy of Higher Education and Research, Jawaharlal Nehru Medical College, Belagavi , Karnataka, India Email:dc.rashmi@yahoo.com
*Corresponding Author

Received: 23.11.2024 Revised: 19.12.2024 Accepted: 20.01.2025

ABSTRACT

Introduction: Dyslipidemias are one of the major modifiable risk factors for coronary heart disease. Globally, 80% of deaths due to cardiovascular diseases occur in low- and middle-income countries. Screening by biochemical analysis in a good quality central laboratory would be ideal, but the cost and safety of sample transportation in cold chain are concerns in a large developing country like India with limited resources. Transportation of samples in the form of dried serum spots would ease blood processing, storage, and transport. In the present study, the stability of cholesterol in dried serum spots on filter paper at room temperature for different time intervals was studied. **Material & Methods:** 100 samples in the laboratory of a tertiary care hospital of south India were analyzed. An aliquot of each serum sample was analyzed immediately and exact 10-[micro] L replicates of the samples were spotted onto 3M Whatman filter paper. After drying, one aliquot was eluted and analyzed on the day of collection. The remaining filter discs were kept in sealed plastic bags and analyzed on 7, 14, 21, 28 and 35 days. Results: Mean and Standard deviation for Cholesterol levels were 147.24+/-30.62. Bland Altman plots showed the differences in values obtained from fresh and dried serum, within two standard deviations for most of the samples. An Intra class correlation coefficient of 0.98 was evident between dried serum spots and fresh serum. Our Study has given the values of Cholesterol from Dried serum spots within medically allowable error. **Discussion**: Dried serum spot collection has advantages compared to conventional blood draws, such as less sample volume is required, convenience of repeating measurements, and easy sample storage and transport. The comparable values between dried serum spots and conventional serum assays supports its usage as an alternative when conventional venous blood draw facilities are not available or accessible. However, precision and accuracy of the results can be improved by opting standard spotting methods and proper storage. The stability, efficient recovery, and excellent correlation with fresh serum samples makes the dried spot assay reliable and convenient method for screening risk factors like cholesterol. Screening patients to identify these risk factors can help prevent cardiovascular diseases and Type 2 Diabetes Mellitus by providing information for early intervention and treatment.

Keywords: Dried serum spots, Whatman filter paper, Cholesterol estimation

INTRODUCTION

Guthrie and Susi introduced the concept of obtaining blood on filter paper in 1963 for the screening of metabolic diseases. The advantages of Dried spot technology over conventional serum assays are as follows, it allows convenient transport of samples and drying of sample destroys infectious viruses, such as human immunodeficiency virus, and ensures sample stability for longer duration at room temperature thereby allowing for transport without the requirement of cold chain or any other special precautions.[1]

Screening of risk factors for non-communicable diseases (NCD) is required as they are fast increasing in the population. The World Health Organization has put up a three-step approach for screening of risk factors: collecting information through questionnaire, carrying out simple physical measurements and collection of blood sample. Due to complexities of the measurement involved, biochemical analysis is not done in all developing countries. Especially in a large country such as India, biochemical analysis at remote corners of the country will be difficult because of the limited availability of resources and technical staff. Carrying out the analysis in a good quality central laboratory is ideal, but the feasibility of sample transportation is a concern. Transportation of samples in the form of dried serum would circumvent the need for blood processing, storage, and shipment at ultralow temperatures. [2]

Total cholesterol has been recognized as important modifiable risk factor for cardiovascular diseases. [3,4,5] Recent studies support emergence of the term "cardiometabolic risk" defined by Watson as "the cluster of modifiable risk factors and markers that identify individuals at increased risk for cardiovascular diseases (myocardial infarction, stroke, peripheral arterial disease) and type 2 diabetes."[6] Globally, 80% of deaths due to cardiovascular diseases occur in low- and middle-income countries. According to the World Health Organisation (WHO) by 2030 more than 23 million people will die annually from CVD. The primary and secondary intervention in developed countries have made CVD a disease of older age, whereas in developing countries the age of onset is at a younger age.[7]

Starck and Lovegren used 4°C stored blood specimens for 5-14 years for the analysis of sterols. They did not look at sterol levels in fresh samples and therefore could not evaluate the degree of degradation with storage.[8]

Kapur et al have studied the use of dried blood for cardiometabolic risk factors, including triglycerides for screening of patients.[1] The collection of samples was done under controlled laboratory conditions.

Quraishi *et al*. have studied triglyceride levels in dried blood spots and have concluded that levels were stable for 30 days at 16–28°C and for 90 days at 4°C .[9]

In the present study we assessed the stability of cholesterol in dried serum spots for 35 days and found that the cholesterol values were in acceptable error range hence the use of dried serum spots for screening cholesterol levels will be helpful in providing information for early intervention and treatment for the individuals at increased risk for cardiovascular diseases.

MATERIALS AND METHODS

A Cross-sectional study was done on the Blood Samples collected for lipid profile at a tertiary care hospital. Considering the mean and standard deviation values from previous studies done, at 95% confidence limit and 5% Tolerance level, the sample size was 83. Considering contamination and loosing results, 100 samples were analysed. Hemolysed samples and Samples of Jaundice patients with high bilirubin were excluded. Approval to conduct the study was obtained from the Institutional ethics committee on human subjects research.

Analysis was done according to the details mentioned by Lakshmy Ramkrishna et al. [10] An aliquot of fresh serum sample was analyzed immediately for Cholesterol levels with the commercially available kit in a semi autoanalyzer. From the remaining serum, exact 10-[micro] L replicates were spotted onto 3M Whatman filter paper kept on a nonabsorbent surface (thermacol) and left at room temperature for 1 h for drying. After drying, one aliquot was eluted and analyzed on the day of collection. The remaining filter discs were kept in a sealed plastic bag to protect them from dust and moisture and stored at room temperature for different time periods.

At 7, 14, 21, 28 and 35 days, entire dried serum spots corresponding to 10 [micro]L were cut out with scissors and transferred to 1.0 ml of enzymatic reagent. For estimation of Cholesterol in fresh serum, 20 [micro] L serum was added to 1 ml of the reagent and the reaction was carried out at 37^{o} C for 10 min. For estimation of Cholesterol in dried serum on filter paper, 2 spots of 10 [micro] L were added to 1 ml reagent and the reaction was carried out for 30 min, according to Lakshmi Ramkrishnan et al 10 min will not be sufficient for the reaction to reach completion. Analysis was done using a Erba semiautoanalyser.

Precautions are needed for proper spotting, complete drying and proper storage to protect samples from dust, moisture and other contaminants. Careful cutting out of sample spots and complete elution of analyte has to be done.

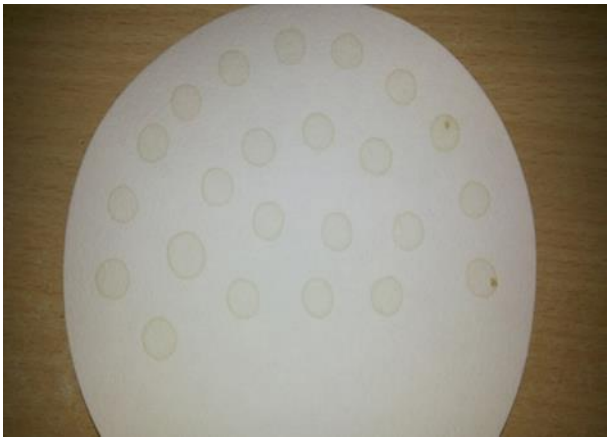
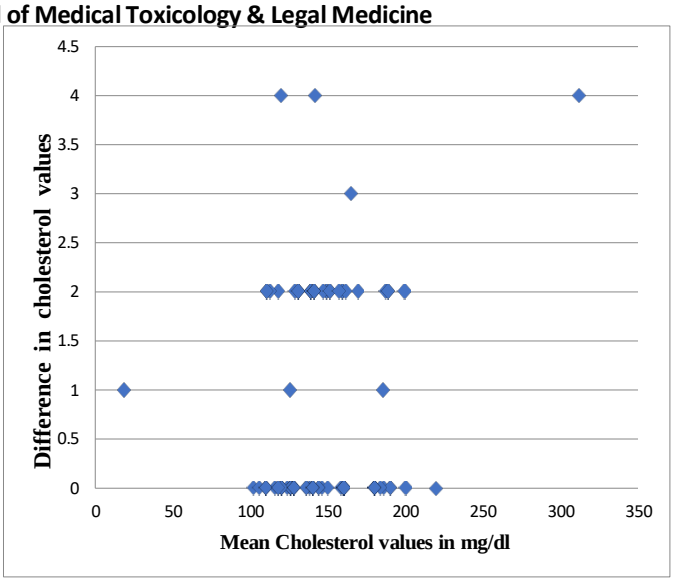


FIGURE 1 - Whatman Filter Paper With Dried Serum Spots

RESULTS

Cholesterol values in the 100 samples analyzed ranged from 102 mg/dl to 314 mg/dl.. The mean +/- standard deviation (SD) cholesterol values obtained from fresh serum was 148.33+/-30.68 mg/dl and the mean cholesterol values from corresponding dried serum was 147.86+/-30.67 mg/dl on the same day of drying and subsequently 147.59+/-30.47 (day 7) , 147.3+/-30.52 (day14) , 146.74+/-30.62 (day 21) , 146.45+/-30.69 (day 28) and 146.41+/-30.66 (day 35). An Intraclass correlation coefficient of 0.98 for cholesterol was evident between dried serum spots and fresh serum. Bland Altman plots were done with Mean of Cholesterol values on x axis against differences in cholesterol values between fresh serum and dried serum on all (0,7,14,21,28 and 35)days. Bland–Altman plots suggest that the difference in values obtained by the two methods was within the 2 SD limits for most of the samples for Cholesterol. Less than 5% of the values were outside the 2 SD limits. [11]

Tonks and Barnett have published estimates of medically allowable error for critical decision levels. Tonks criteria are general (smaller of one fourth of the normal range or 10 % of the measured value. Barnetts recommendations are widely used but are limited to common laboratory analytes. Westguard has transformed Barnetts limits to 95% limits of allowable error i.e 95 % of patients should have errors less than the limit , or only 1 of every 20 samples can have an error larger than the specified limit.[12] Our Study has given the values of Cholesterol from Dried serum spots within medically allowable error.



GRAPH 1 – BLAND ALTMAN PLOT OF CHOLESTEROL VALUES FROM FRESH SERUM Vs DRIED SERUM SPOTS

DISCUSSION

Despite the clear benefits by Dried serum sampling, researchers are hesitant to adopt the method for various reasons. The first is unpredictability: quite simply, fear of the unknown. The second reason is status quo bias: researchers are uncomfortable changing a method that is already working. The third reason is data comparability loss: concern for discrepancies between historic data vs new data. Finally, the lack of clear guidelines.

The analysis of dried serum spot samples compared to conventional serum analysis have the following differences. As the sample is dried, analytes have to be brought into solution. The serum spots are cut out with scissors and put into an elution reagent for a fixed amount of time. Whole blood comprises liquid and cellular fractions, and centrifugation of samples removes cellular components to yield serum or plasma. (Serum and plasma are comparable in this regard: the difference is that clotting factors have also been removed in serum.) When blood samples are dried on filter paper, cellular elements get ruptured, and their components are released into solution when they are reconstituted. Different analytes will vary in their sensitivity to potential interference, and some assays may require additional processing prior to analysis being done.[13]

Investigators can expect performance that is comparable to that obtained with conventional samples, which may not always be possible. In such scenario, the benefits of DBS for collection of sample and transportation have to be compared against the conventional methods. Additional procedures for extraction are required for certain analytes to overcome this problem. Elution of the analyte from the sample and volume of sample collected are two other issues of concern in analysis with dried spots.

Minimization of preanalytical variations with proper collection of serum spots and storage are important determinants for the success of mass screening of total cholesterol using Dried serum spots for risk factor assessment. Furthermore, many standard clinical assays are performed on automated, high-throughput analyzers designed for use with serum or plasma samples. These instruments offer increased speed and reduced costs of analysis but currently are not likely to accommodate dried serum samples.[14]

Serum can be stored at -20°C in a non-self-defrosting freezer for up to 4 weeks. For longer storage (> 4 weeks) they should be maintained at -80°C or lower. Total cholesterol levels are stable for at least one year at -80ºC or lower. Whereas dried serum spots can be stored at room temperature for up to 35 days, according to our study.

The stability of dried serum samples for cholesterol at room temperature for 35 days promises a great alternative in developing countries with large rural population and limited availability of laboratory facilities. Further, the method will be useful for multicentric studies where the cost and safety of sample transportation to a distant laboratory are limiting factors.

REFERENCES

1. Kapur S, Kapur S, Zava D. Cardiometabolic risk factors assessed by a finger stick dried blood spot method. Journal of diabetes science and technology. 2008 Mar;2(2):236-41.
2. Lakshmy R, Gupta R, Prabhakaran D, Snehi U, Reddy KS. Utility of dried blood spots for measurement of cholesterol and triglycerides in a surveillance study. 2010 Mar 1: 258-262.
3. Sprecher DL. Triglycerides as a risk factor for coronary artery disease. The American journal of cardiology. 1998 Dec 17;82(12):49U-56U.
4. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease--the Framingham Heart Study. The Canadian journal of cardiology. 1988 Jul;4:5A-10A.
5. Dennis LS. Triglyceride as a risk factor for coronary artery disease. Am J Cardio. 1998;82:49U–59U.
6. Watson K. Managing cardiometabolic risk: an evolving approach to patient care. Critical pathways in cardiology. 2007 Mar 1;6(1):5-14.
7. McDade TW, Burhop J, Dohnal J. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. Clinical chemistry. 2004 Mar 1;50(3):652-4.
8. Starck L, Lövgren A. Diagnosis of Smith–Lemli–Opitz syndrome from stored filter paper blood specimens. Archives of disease in childhood. 2000 Jun 1;82(6):490-2.
9. Quraishi R, Lakshmy R, Prabhakaran D, Mukhopadhyay AK, Jailkhani B. Use of filter paper stored dried blood for measurement of triglycerides. Lipids in health and disease. 2006 Jul 14;5(1):20.
10. Ramakrishnan L, Reddy KS, Jailkhani BL. Measurement of cholesterol and triglycerides in dried serum and the effect of storage. Clinical chemistry. 2001 Jun 1;47(6):1113-5.
11. Bland JM, Altman DG. Measuring agreement in method comparison studies. Statistical methods in medical research. 1999 Apr;8(2):135-60.
12. Bishop M , Duben Von Laufen J , Fody E Clinical Chemistry Principles, Procedures, Correlations ; 1985: 74
13. Ahluwalia N. Diagnostic utility of serum transferrin receptors measurement in assessing iron status. Nutrition reviews. 1998 May 1;56(5):133-41.
14. McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography. 2007 Nov 1;44(4):899-925.