

Measurement of Creatinine from Dried Blood spot by Enzymatic Method

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Abstract: *Background: DBS on filter paper has been shown as a feasible matrix for collection, transport and analysis of biochemical analytes. Creatinine, is used as a marker of renal function and staging chronic kidney disease (CKD), often requiring repeated testing for drug dosing in patients who undergo dialysis or those who have had arenal transplant. Blood collected and dried on filter paper would be relatively less invasive in such patients for measurement of creatinine.*

Method: Blood spots made on filter paper were used for creatinine estimation by enzymatic method and compared with measurement in plasma. The effect of storage was assessed on stability of creatinine in DBS.

Result: A good correlation was evident between plasma and dried blood on the day of collection and was stable till one week at room temperature as well as 4°C.

Conclusion: The study demonstrates feasibility and stability of dried blood for creatinine estimation. The method has the advantage of being less invasive and relatively painless.

Keywords: *Creatinine, Dried Blood Spot, epidemiological studies, kidney function, filter paper, enzymatic method*

1. INTRODUCTION

Creatinine is the most common indicator of kidney function. The ideal indicator of kidney function is considered glomerular filtration rate (GFR), GFR cannot be measured directly, but instead it can be assessed by the renal clearance of filtration markers., such as inulin, iohexol, iothalamate, and Cr - EDTA, these procedures are costly and time consuming and are not suited to the routine detection of kidney disease and not applicable for field studies. The clearance of endogenous substances, such as urea and creatinine, can be measured but requires both serum and an accurately timed urine collection, hence efforts have been directed at more convenient "urine-free" estimates of GFR[1]. The GFR can be determined from the renal clearance of a marker that achieves stable plasma concentration, is inert , and is freely filtered by the glomeruli but not reabsorbed, secreted, or metabolized. The search for such an endogenous marker is going on. Creatinine, one of the clinically useful analytes has long been used by clinicians as a marker of renal function. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass.

Currently, clinical laboratory diagnosis is carried out through measurement of biochemical analyte in whole blood, serum and plasma. Dried blood spots (DBS) prepared from peripheral blood have been a successful matrix for epidemiologic screening. In principle, any analyte that can be measured from whole blood, serum, or plasma can also be measured from DBS. This method, in which finger-stick blood, collected onto special filter paper and then dried and later eluted for testing, has been used for analysis of hormones [2], lipids [3], and therapeutic drugs [4], as well as for genetic screening [5]. DBS samples have played central roles in disease-surveillance efforts in several developing countries and have facilitated research on human biology and health in remote settings around the world [6-8]. Large-scale epidemiological surveys can be performed rapidly, enabling strategic decisions to be made about targeting at risk populations. The high cooperation rate and quality of the spots collected

in the National Social Life, Health, and Aging Project in US suggest that the collection of DBS in population-based research is a feasible and viable alternative to venous blood draws [9].

The method has the advantage of being less invasive, relatively painless, particularly suitable for collection in neonates and the elderly and most suitable in field studies. Repeated estimation of renal function is often required for drug dosing or patient care especially for patients who have undergone transplant or dialysis. The steady aging of the population and the increase in illness severity of hospitalized patients means collection of blood at regular intervals. Phlebotomy in elderly patients is a challenge due to several changes that occur during the normal aging process. Skin changes like dermal thinning, waning elasticity and de-pigmentation and blood vessel changes like fragile subsurface blood vessels and atherosclerotic narrowing of the vessels make the venipuncture difficult. Field studies are also being carried out to assess magnitude of CKD in community. Collection of blood on filter paper through a finger prick is a relatively non invasive option for recurrent analysis.

To the best of our knowledge measurement of creatinine from dried blood has not been reported. This study reports the use of dried blood spot (DBS) for the measurement of creatinine.

2. METHOD

Patients (N=25) visiting the Nephrology department of the All India Institute of Medical Sciences, New Delhi, India, for routine creatinine evaluation were selected at random. Blood was collected by venipuncture into tubes with sodium fluoride as an anticoagulant. All procedures were in accordance with the ethics standards of our institution. Blood spots were prepared on the Whatman filter paper (No. 3) kept on a non adsorbent thermacol surface and were kept at room temperature for drying. After drying, the filter discs were kept in sealed plastic bags to protect from dust and moisture and stored at 4 °C. The plasma was separated and directly analyzed on the day of collection for comparison. The dried blood spots were analyzed on the same day as well as one week later. The demographic details were not collected as this was a validation study only.

For creatinine measurement from dried blood, two disks (6 mm diameter) was punched out and put in a tube with a Teflon screw cap. One hundred microliters of methanol (analytical grade, Qualigens, Glaxo Limited, India) was added to the tube. Tubes were incubated at 37°C for 2 hours, with intermittent mixing on vortex shaker [10]. To minimize matrix differences and maximize comparability between calibrators and test samples, dried blood spot standards and controls were prepared by mixing with washed red blood cells. The quality control material was diluted serially in normal saline, and washed erythrocytes were mixed in 50:50 (v/v) proportions to get whole blood calibrators. Blood-based quality controls were prepared similarly by adding washed erythrocytes in 1:1 dilution

Plasma creatinine was measured by enzymatic method (Dialab, Austria) that involves a series of coupled enzymatic reactions which include; creatininase enzymatic conversion of creatinine into the product creatine, which itself is converted to sarcosine by creatinase. Sarcosine undergoes oxidation to sarcosine oxidase producing hydrogen peroxidase. In the presence of peroxidase the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye. Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

3. STATISTICAL ANALYSIS

The creatinine values obtained in fresh plasma and in DBS at day 0 and day 7 was compared by Pearson correlations. The Intra-Class Correlation Coefficient was computed to measure the reliability and validity within a set of data. Bland–Altman plots were prepared to ascertain bias. The paired sample t-test was done to determine whether there is a significant difference between the average values of the same measurement made under two different conditions. $P < 0.05$ was considered significant. All the statistical analysis were carried out using SPSS version 16.0 (SPSS Inc., Chicago, IL)

4. RESULTS

The mean [standard deviation (SD)] creatinine value obtained from plasma was 1.35 (0.5) mg/dl and the mean creatinine from corresponding dried blood spot was 1.39 (0.46) mg/dl. Figure 1 shows the scatter plot of all values of creatinine obtained from dried blood and plasma at day 0. A correlation coefficient of $r=0.92$ was evident between dried blood and plasma whereas the Intra Class Correlation (ICC) for the same was 0.94. The correlation coefficient between plasma and dried blood stored for

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one week at room temperature (N=25) as well as 4°C (N=15) was $r=0.91$ and the ICC were 0.89 and 0.92 respectively. Paired t-test carried out between plasma and dried blood spot at day 0 ($p<0.29$), plasma and dried blood stored for one week at room temperature ($p<0.14$) as well as 4°C ($p<0.91$) showed that the differences were not significant. 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 (Figure 2 and figure 3).

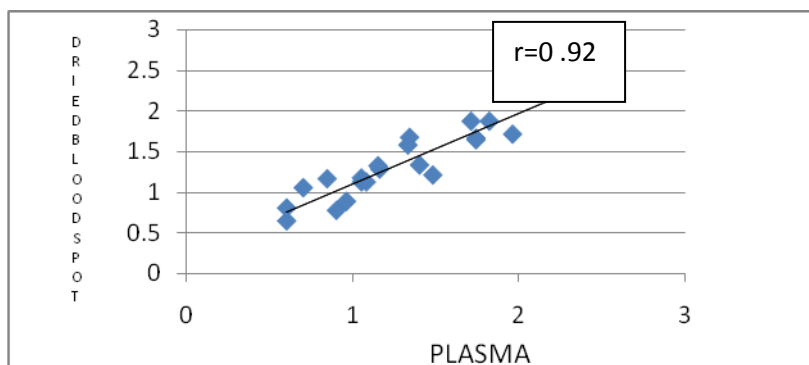


Figure1. Scatter plot of creatinine from plasma and dried blood at day 0.

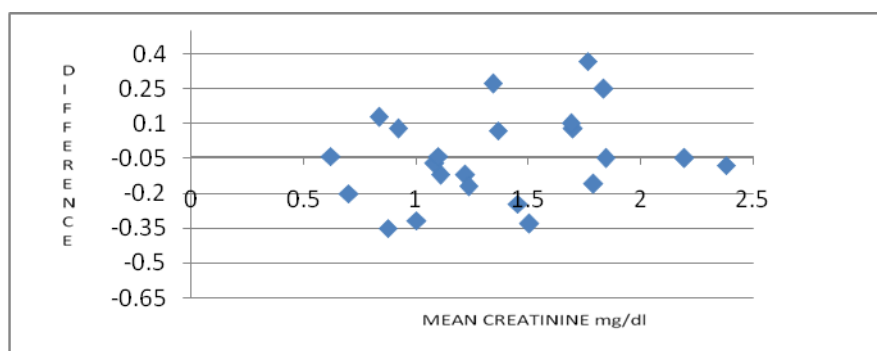


Figure2. Bland–Altman plot of differences in creatinine values between dried blood and plasma at day 0

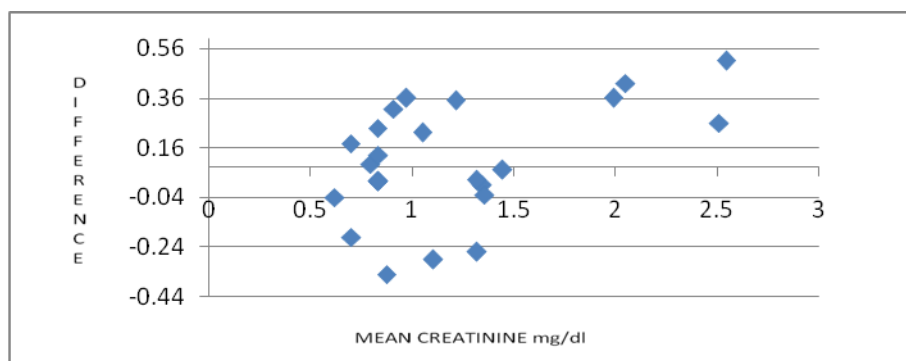


Figure3. Bland–Altman plot of differences in creatinine values between dried blood and plasma at day 7 at room temperature

5. DISCUSSION

The collection of DBS on filter paper offers a powerful tool in screening programs and in patients who require repeated sampling [11], a dermal puncture is preferred for dried blood spot over venipuncture where too much blood may be inadvertently collected. Renal function is regularly monitored to reduce diabetes complications by measuring microalbumin and creatinine. The National Kidney Disease Education Program recommends calculating glomerular filtration rate from serum creatinine concentration. The creatinine clearance test is used to monitor the progression of renal disease. These studies have relevance for the advancement of analytical method development and public health knowledge [12].

The creatinine levels obtained in plasma samples were compared with paired dried blood samples on the day of collection. A good correlation between measurement of these analytes in dried blood spot and plasma suggests that the method is valid and has the potential to be used for the screening. The

values obtained by the two methods correlated well with the r value of 0.92 and intra class correlation value of 0.94. To assess the stability of creatinine in the DBSs, filter paper discs were punched from DBSs, and the creatinine level of DBS stored at 4°C and room temperature was estimated at 7th day. We found that the creatinine levels remained stable in DBSs kept at 4°C and 37°C, respectively.

Rizwana et al have demonstrated the stability of filter paper as a feasible matrix for collection and transport of serum for creatinine measurement [13] but we have carried out the study in whole blood circumventing the need for centrifugation and more applicable for finger prick as is the case for preparation of DBS. Creatinine measurement from DBS was reported by Koop et al [14] recently using liquid chromatography-tandem mass spectrometric (LC-MS/MS) technique, which is an expensive technique requiring trained personnel and specialized instrument. The measurement of creatinine in urine dried on filter paper have been reported by Zavaet et al (15).

An advantage of this study is the use of enzymatic method for creatinine estimation, which is more specific than routine Jaffe assay as they give falsely high results [1]. Unlike venipuncture, the collection of capillary blood either through heel or finger prick is relatively painless and noninvasive, particularly in infants and the elderly. Dried blood is suitable for field or home collection as it circumvent the need for centrifugation, separation of samples and the need for maintaining a cold chain for transportation of the sample. Blood spots further represent a low infectious hazard, as many viruses known to be present in serum or plasma lose infectivity as a consequence of disruption of their envelope on drying [16]. A limitation of the study is the inability to compare all the samples at the different time points/ temperature, primarily due to low number of dried blood spot that were made.

Conclusion: We conclude that creatinine is highly stable in dried blood spot and is readily transferable to a liquid phase for analysis.

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