Creatinine Measurement and Stability in Dried Serum

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Studies have shown an association between serum creatinine levels and type 2 diabetes in various populations.[1–2] For epidemiological studies and screening programs, use of a filter paper matrix circumvents transportation issues and has been reported for many analyses.[3–6] However, the use of dried blood or serum has not been reported for the measurement of creatinine. This study reported the use of dried serum for the measurement of creatinine.

For the study, 60 leftover serum samples from the subjects' visiting department of laboratory medicine, All India Institute of Medical Sciences, were collected for laboratory investigations. Ethical clearance for the conduct of the study was obtained from institutional ethics committee. An aliquot of each serum sample was analyzed immediately. Remaining aliquot was spotted on filter paper. Multiple spots were approximately 10 µl, and each was prepared on 3 micron Whatman™ filter paper (Whatman plc., Kent, United Kingdom). After drying, the discs were kept in sealed plastic bags and stored at 37 ˚C and at 4 ˚C.

For serum creatinine estimation, 100 µl of the serum was added to 1 ml of the colorimetric reagent (Randox Laboratories Ltd., Crumlin, United Kingdom). For dried plasma creatinine estimation, five (6 mm) discs were punched from the prepared spots and dropped into 10 ml glass tubes containing 1 ml of the colorimetric reagent. For fresh serum, the reaction time was 10 min. The extraction from dried serum was carried out for a period of 30 min, with gentle shaking at 100 rates per min in an environ shaker (Lab-Line Instruments Inc., Melrose Park, IL) at 37 ºC. The final absorbance was read at 540 nm wavelength. Calibration was done with calibrators spotted onto filter paper and treated the same way as samples.

Total creatinine values in the 60 samples (fresh) ranged from 0.5 to 3.3 mg/dl. The creatinine levels obtained in serum samples were compared with paired dried serum samples on the day of collection. Creatinine mean (± standard deviation) values in serum and their dried serum values were 1.99 (± 0.64) and 1.92 (± 0.55) mg/dl, respectively. The values obtained by the two methods correlate well with the *r* value of 0.94 and intraclass correlation value of 0.93 with 96% recovery on the day of sample collection.

To assess stability of the dried serum samples, discs were punched from a subsample size (n = 15), and creatinine levels were estimated at the end of 0, 15, 30, 60, and 90 days. Mean creatinine value in dried serum eluted and measured on the day of collection (day 0) did not differ significantly from other storage days. Storage of filter disc at 37 ˚C and at 4 ˚C for 15, 30, 60, and 90 days did not significantly alter the mean creatinine values (**Table 1**).

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Table 1.
Effect of Storage of Dried Serum on Creatinine Concentrations

Days of storage (temperature)	Dried serum creatinine (mg/dl)
0	2.11 ± 0.97 (Serum)
15 (37 °C)	1.97 ± 0.89 (Dried serum)
30 (37 °C)	2.06 ± 0.99 (Dried serum)
60 (37 °C)	2.09 ± 0.99 (Dried serum)
90 (37 °C)	2.13 ± 0.92 (Dried serum)
90 (4 °C)	2.17 ± 0.97 (Dried serum)

This study demonstrates filter paper as a feasible matrix for collection and transport of serum for creatinine measurement. This approach needs to be evaluated in whole blood also. The stability of creatinine in a filter matrix for up to 3 months makes this method very promising for epidemiological studies.

We conclude that creatinine is highly stable in dried serum and is readily transferable to a liquid phase for epidemiological studies.

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References:

1. Harita N, Hayashi T, Sato KK, Nakamura Y, Yoneda T, Endo G, Kambe H. Lower serum creatinine is a new risk factor of type 2 diabetes: the Kansai healthcare study. Diabetes care. 2009;32(3):424–6. Epub 2008 Dec 15.
2. Hjelmesæth J, Røislien J, Nordstrand N, Hofsø D, Hager H, Hartmann A. Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study. BMC Endocr Disord. 2010;10:6.
3. Howe CJ, Handelsman DJ. Use of filter paper for sample collection and transport in steroid pharmacology. Clin Chem. 1997;43(8 Pt 1):1408–15.
4. Ramakrishnan L, Reddy KS, Jailkhani BL. Measurement of cholesterol and triglycerides in dried serum and the effect of storage. Clin Chem. 2001;47(6):113–5.
5. Quraishi R, Lakshmy R, Prabhakaran D, Mukhopadhyay AK, Jailkhani B. Use of filter paper stored dried blood for measurement of triglycerides. Lipids Health Dis. 2006;5:20.
6. Quraishi R, Lakshmy R, Prabhakaran D, Irshad M, Mukhopadhyay AK, Jailkhani BL. Effect of storage temperature on cholesterol measurement from dried blood. Indian J Med Res. 2007;126(3):228–9.