Evaluation of stability of urea and creatinine in dried blood spot stored at different temperature

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Abstract

Background: The wide applications and merits of Dried Blood Spot (DBS) sampling has a high significance of early diagnosis and treatment of renal diseases in the context of Nepal where incidence of high mortality and morbidity rate is seen due to lack of proper diagnostic system and medical resources. Urea and creatinine are the easiest parameters to assess renal function test via DBS sampling.

Method: An experimental based, convenient sampling technique was performed during the health camp at Janamaitri Hospital, Kathmandu, Nepal from May 2018 to October 2018. A total of 25 participants were enrolled and blood samples were collected then DBS preparation was done. The serum sample on the day of collection was estimated for Urea and Creatinine concentration whilst DBS were stored under room temperature, 37ºC and 4ºC and examined for both analytes on Day 15, 30 and 45 respectively.

Results: Out of total 25 samples, the mean (± standard deviation) value of urea and creatinine of serum on the day of collection were 25.36 (± 2.90) and 1.03(± 0.16) respectively. The stability was reported to be good at 4ºC for both analytes where *p*-value was non-significant (≥0.05) reflecting its stability whereas under 37ºC, DBS stability in all days declined for Urea with a significant association i.e. $p < 0.05$ reflecting its instability. Mean creatinine values were stable comparatively to that of urea under all temperature and test days. As the storage temperature increases, the decline in concentration of analytes was noted.

Conclusion: This study demonstrated that DBS technique can be used to assess renal function tests and stability of creatinine is suitable under all temperature for longer storage. Optimal temperature for storage to be 4ºC.

Keywords: DBS, urea, creatinine, stability, rural setting

Introduction

In the present era, achieving an accessible health test for any given population is a prominent aspect of health studies. Provided that the clinical tests are accurate and precise, any means of making health screening tests economical and accessible has to be regarded in a positive light [1]. Discovery attributed to Ivar Christian Bang [2], Dried Blood Spot (DBS) can be used for any biochemical parameters that can be measured from whole blood, plasma or serum [3]. Here, blood is drawn and transferred into a cellulose or polymer card paper, air dried for several hours, stored in low gas-permeability plastic bags with desiccant and later eluted for testing [4-5]. Basically, DBS sampling in developing countries has a simplified logistics for remote sampling compared with vein-puncture for minimum human resources, small blood volume, non-invasive method (direct heel-prick/finger-prick), simple collection of non-blood bio fluids such as saliva, easy shipment and disposal and bio-banking for retrospective analysis [6-8].

Health is declared as the fundamental right of human kind by World Health Organization (WHO). Regardless of this fact, many developing nations like Nepal is deprived of even basic health care services. Reasons may be due to its uneven topography, limited resources, lack of proper plans and policies and irregular monitoring has been influencing the early diagnosis of the disease leading to increased morbidity and mortality rate. Under such conditions, with minimal number of health care professionals, it becomes difficult even to carry out the routine tests [4]. Thus, DBS sampling enables the transportation of samples from remote areas to equipped laboratory by regular mail with no risk of contamination compared with conventional sampling [9]. A study conducted by National Public Health laboratory, Kathmandu in between October and December 2009 illustrated that the sample transported in DBS cards have an optimal mean postal delivery duration of 2.25 days, stability for a month at room temperature with sensitivity and specificity of Human Immunodeficiency Virus (HIV) rapid 1 and 2 to be 88.9% and 94% respectively [10].

Researchers have become quite optimistic regarding the potential clinical applications of DBS [11]. Urea or creatinine measurement aids to assess renal function in serum as both reflect glomerular filtration rate that defines kidney function test [12]. A study demonstrated that urea concentrations in DBSs were stable for up to 120 and 90 days stored under 4°C and 37°C, respectively [13]. Another parameter to access renal function is creatinine. A study for creatinine stability elucidated that its DBS sampling showed good correlation between serum and dried blood on the day of collection being stable till one week at room temperature as well as 4°C [14].

According to WHO (2018), mortality rate due to renal disease in Nepal reached 2.74% of total deaths ranking Nepal 53rdin

the world [15]. In Nepal, the overall prevalence rate of chronic Kidney Disease was 6.0% [16]. Therefore, an imperative investigation for the prevalence of renal diseases is required in context to Nepal from every population group. Such that, sampling via DBS from remote areas deprived of sophisticated infrastructures and resources may aid in early diagnosis, minimize morbidity and mortality rate in Nepalese population.

2. Materials and Methods

2.1 Study design and site

This is an experimental study in which convenient sampling was performed in the visiting participants at Janamaitri Hospital, Kathmandu, Nepal between May 2018 and October 2018. Laboratory experiments were conducted in the Department of Medical Laboratory Technology, JF Institute of Health Sciences (JFIHS), Hattiban, Lalitpur.

2.2 Study population and criteria

A total of 25 blood samples were collected from the participants during health camp at Janamaitri Hospital organized by JFIHS. Those participants with consent and willing to participate were listed in the inclusion criteria whereas those who were not interested to participate were enlisted in exclusion criteria.

2.3 Sample collection and processing

Initially, 3ml of venous blood was collected from the participants with sterile syringe and transferred in a gel tube vial. DBS samples were prepared immediately after the collection of blood before it clotted. For DBS preparation, 50µL of whole blood was pipette out and transferred onto the filter paper to make a dried blood spot. This step was repeated two more times to make at least three DBS of each sample. Finally, serum was separated from the blood by centrifuging the sample for about 10 minutes at 5000 rpm. For the measurement of Urea (Enzymatic method) and Creatinine (Jaffe's Method), firstly, serum sample was used to estimate their respective concentration via Stat Fax, semi-automated analyzer and allocated as concentration at '0' day. DBS samples were then stored at room temperature, 37°C and 4°C.and their respective concentrations were measured via elution of DBS at the regular intervals of 15 days, 30 days and 45 days and noted down.

2.4 Statistical Analysis

Statistical Package for the Social Sciences version 21 was used for data entry and encoding the participants. Calculation of Mean and Standard Deviation was done for each set of tests. Similarly, *p*-value and C.I. were estimated with significant level of *p*-value<0.05.

2.5 Validity and reliability of the tests

Quality/Reference procedure was employed with appropriate controls/standards.

3. Results

Here, in this study a total of 25 samples were collected to measure the stability of DBS sampling for urea and creatinine level. The result showed that the mean (standard deviation) value of urea and creatinine of serum on the day of collection were 25.36 (2.90) and 1.03(0.16) respectively. The mean value of Urea was comparatively stable at 4ºC in test days of 15 and 30. However, value tend to slightly decrease in all temperature i.e.RT, 4ºC and 37ºC after 45 days. Meanwhile, it was noted that all the mean value of urea fluctuated at 37ºC in all test days. For the mean value of creatinine level, the stability comparatively corresponds to that of urea where its level at 4ºC in all test days was nearly similar to day 0 value with a slight decrement though. The mean values were in the decreasing order with the increasing days inferring that its stability decreased with time. At 37ºC the stability varied widely than the day 0.

Table 1 infers the comparison of mean (SD) of urea level at different temperature on different test days via *p*-value and CI which showed that all the values were significantly associated with $p < 0.05$ in all test days at temperature RT, 37ºC and 4ºC except for DBS card stored at RT (*p*-value = 0.09, CI= -2.95 - 0.23) and 4ºC (*p*-value = 0.32, CI= -2.42 - -0.82) on day 15 where the mean value was non-significant. That means the urea level on day 15 at those temperatures were stable.

Table 1: Comparison of mean (SD) of Urea at different temperature on day 0, 15, 30 and 45

Day	Urea at RT			Urea at 37ºC			Urea at 4ºC		
	Mean ± S.D.	***p*-value**	**CI**	**Mean ± S.D.**	***p*-value**	**CI**	**Mean ± S.D.**	***p*-value**	**CI**
0	25.36 ± 2.90	Ref.	Ref.	25.36 ± 2.90	Ref.	Ref.	25.36 ± 2.90	Ref.	Ref.
15	24.00 ± 2.67	0.09	-2.95 -0.23	22.64 ± 2.43	0.0008	-4.24 - -2.20	24.56 ± 2.78	0.32	-2.42 - -0.82
30	22.68 ± 2.73	0.0015	-4.28 - -1.08	20.64 ± 2.69	<0.0001	-6.31 - -3.13	23.52 ± 2.72	0.03	-3.44 - -0.24
45	21.28 ± 2.79	<0.0001	-5.70 - -2.46	18.92 ± 2.59	<0.0001	-8.00 - -4.88	22.36 ± 2.54	0.0003	-4.55 - -1.45

Here, **Table 2** represented the comparison of mean (SD) of creatinine level at different temperature on different test days calculating *p*-value and CI. It showed that all the mean values were non-significant (p-value ≥ 0.05) that is the creatinine measurements at all given temperatures on different test days are relatively stable on DBS sampling.

Table 2: Comparison of mean (SD) of Creatinine at different temperature on day 0, 15, 30 and 45

Day	Creatinine at RT			Creatinine at 37ºC			Creatinine at 4ºC		
	Mean ± S.D.	*p*-value	CI	Mean ± S.D.	*p*-value	CI	Mean ± S.D.	*p*-value	CI
0	1.03 ± 0.16	Ref.	Ref.	1.03 ± 0.16	Ref.	Ref.	1.03 ± 0.16	Ref.	Ref.
15	1.00 ± 0.16	0.51	-0.12-0.06	0.99 ± 0.16	0.38	-0.13 - -0.05	1.01 ±0.16	0.66	-0.11 – 0.07
30	0.99 ± 0.16	0.38	-0.13 -0.05	0.97 ± 0.16	0.19	-0.15–0.03	1.00 ±0.16	0.51	-0.12 – 0.06
45	0.97 ± 0.16	0.19	-0.15 - 0.03	0.95 ± 0.16	0.08	-0.17 – 0.01	1.00 ±0.16	0.51	-0.12 – 0.06

Figure 1. depicted that the coefficient of variance (CV) value of Urea showed a maximum variation on day 45 at 37ºC and similar value of CV at RT on day 30 whereas.

Figure 2 showed that CV of creatinine varied largely at day 45 at 37ºC and same value on day 15 at 4ºC.

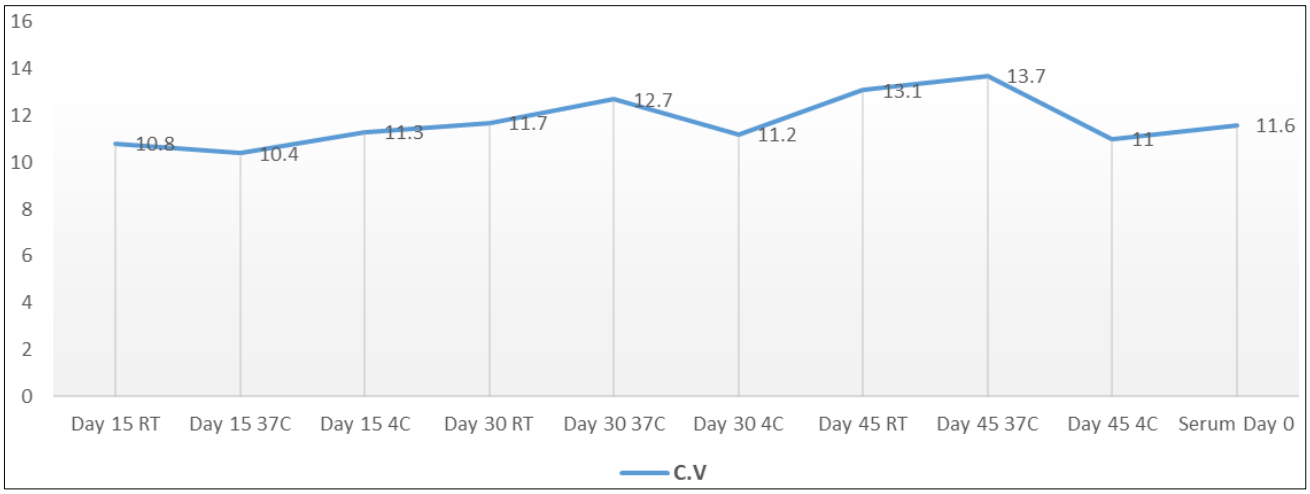

Fig 1: A line diagram presenting the C.V of Urea concentration observed on DBS storage under different temperature conditions on different days.

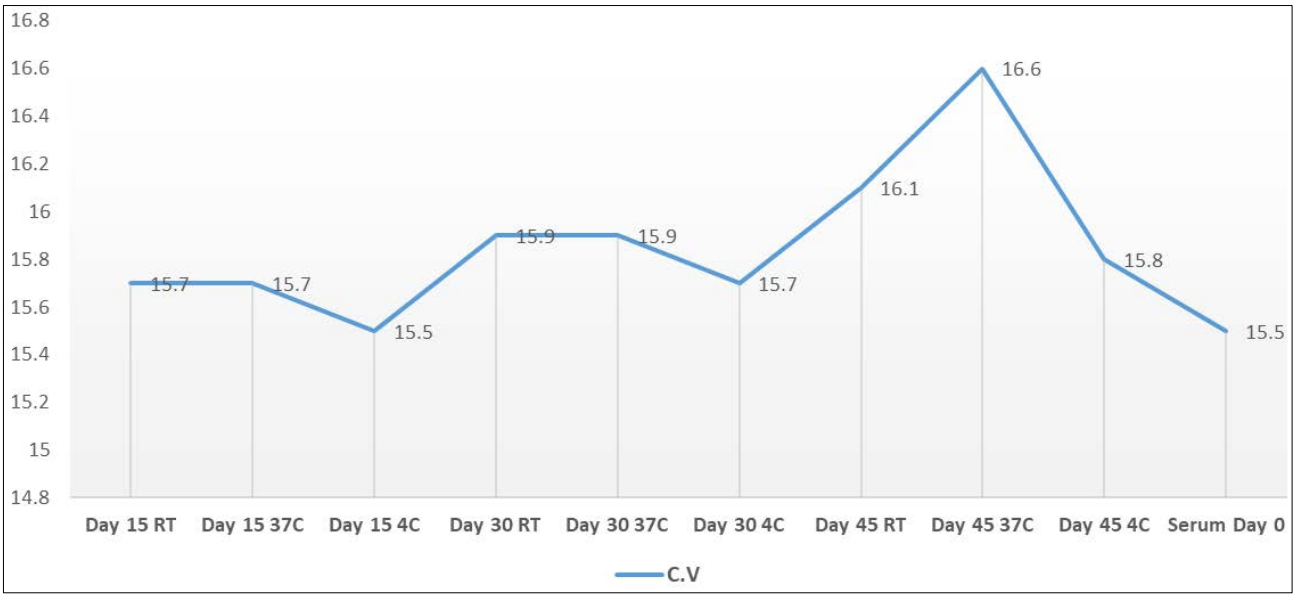

Fig 2: A line diagram presenting the C.V of Creatinine concentration observed on DBS storage under different temperature conditions on different days.

4. Discussion

In this trend of development of precision medicine, tools that contribute for easy sampling and enhancing bioanalytical empowerments are of utmost need. The CDC mentioned on the quality of DBS collection, noting that "The filter paper blood collection device has achieved the same level of precision and reproducibility that analytical scientists and clinicians have come to expect from standard methods of collecting blood, such as vacuum tubes and capillary pipettes, further lending credibility for the use of DBSs in clinical analysis"[17].Worldwide, the elevating prevalence of many chronic diseases such as diabetes mellitus, hypertension, cardiovascular and renal diseases together with the enhanced medical care have all prioritized-on organs functions test, most importantly renal function. Chronic kidney disease is a prominent risk factor for vascular disease and early cardiovascular mortality as well as progression of kidney disease [18].

Here, in this study urea and creatinine level in serum on day 0 was compared with DBS eluted sample on day 15, 30 and 45 under RT, 4˚C and 37˚C. Urea concentration was relatively stable at 4˚C and RT on day 15 and 30 but value slightly declined after 45 days which may be due to degradation of urea. At 37˚C, the stability of DBS depleted majorly in all test days. We observed the stability till 45 days. However, a study in India revealed that the urea concentration was stable for till 120 and 90 days in DBSs under 4˚C and 37˚C, respectively [13]. Comparing the stability of urea from both studies, it can be said that under 4˚C stability remains consistent even after 45 days but at 37˚C, it showed decline in stability simultaneously. An in-vitro analysis showed that the stability of urea decreases by increase in temperature for all pH values [19]. This may be the reason behind declination of urea concentration at 37˚C.

Our study revealed that creatinine level remains stable under 4ºC temperature. Stability can be considered even under RT in all days but DBS stability slightly changes after 15 days in 37ºC. Eventually, *p*-value (>0.05) remains non-significant for all temperature until 45 days. Thus, it can be said that stability of creatinine is comparatively stable under all temperature than that of urea. Another study carried out in India found that the creatinine levels remained stable in DBSs kept at 4˚C and 37˚C, respectively after a week [3]. The difference in uniformity of creatinine concentration in these study may be that we used Jaffe's method for measuring the concentration which gives falsely high results than enzymatic method with better performance [19-21].

In the context of CV for both urea and creatinine, the CV value peaked up on 45 days under 37ºC. The reasons behind may be due to degradation of urea and creatinine concentration after long days of storage and other studies have also revealed that the stability degrades at higher temperatures [19].

Thus, there is increasing interest in using DBS cards to extend the reach of global health and disease surveillance programs to hard-to-reach populations. Conceptually, DBS offers a cost-effective solution for multiple use cases by simplifying logistics for collecting, preserving, and transporting blood specimens in settings with minimal infrastructure [6]. For all these reason, DBS sampling can be best used for analysis of bioanalytes if stored at optimum temperature, for optimum days using appropriate test procedure.

5. Conclusion

In Nepal, where remote population are still deprived of a primary health-care services, keeping under considerations the advantages of DBS sampling and transportation under optimal condition, renal function tests specifically creatinine and urea concentration can be easily measured using this technique and early diagnosis, treatment and surveillance of renal diseases can be promptly done.

Abbreviations

CDC: Centers for Disease Control and Prevention
CV: Coefficient of Variance
DBS:Dried Blood Spot
p-value: Probability value
RT: Room Temperature
S.D.: Standard Deviation
SPSS: Statistical Package for the Social Sciences

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