# Stability Study of Urine Creatinine Levels in Filter Paper Based Method Transport Medium

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**Abstract: -** Kidney disease is a major health problem worldwide, and creatinine is one of its markers for diagnosis. Alternative methods for the measurement of creatinine have been developed, including urine dried on filter papers. Studies proved its accuracy and reliability in comparison with conventional methods involving liquid urine. The researchers aimed to study the stability of human urine creatinine stored in various containers under different temperatures and durations. Urine samples from three (3) participants were subjected to eight (8) combinations of duration, temperature, and container, then triplicated to a total of 72 dried urine samples (DUS). Creatinine was measured using elution and reagent strip analysis. Findings showed that any combination of the physical factors could be used to store and transport DUS. There were no significant differences in the creatinine between one month and one week durations [t (70) = -0.801 ($p > 0.05$)], 25°C and 4°C [t (70) = 1.209 ($p > 0.05$)], and ziplock and microwavable plastic containers [t (7) = 1.209 ($p > 0.05$)]. The combination of one-week duration under 4°C in microwavable plastic containers was the most stable, having the lowest mean difference (0.0 mg/dL). Storage of one month under 4°C in ziplocks showed the largest mean difference of 22.22 mg/dL, representing the least stable combination. The addition of physical variables, procurement of DUS from a broader range of participants with normal and impaired kidney functions, and usage of quantitative creatinine assay to eliminate subjective biases and increase the specificity of the method are recommended.

**Key Words: —*Creatinine, dried urine, duration, temperature, container*.**

## I. Introduction

"A hidden epidemic" is what renal experts say about the worldwide issue of kidney disease (Preidt, 2018) [1]. This major health problem affected 850 million people globally and is recorded to be the 7th leading cause of death among Filipinos. The National Kidney and Transplant Institute stated that the cost of treatment for kidney disease is quite expensive. As a major public health concern, a convenient and practical test is needed to diagnose and treat kidney diseases as early as possible.

Feher (2017) stated that creatinine is a waste product in the blood produced by the body during muscle metabolism. It is filtered by the kidneys, and excreted in the urine, and serves as a marker for kidney problems through analysis in blood and urine samples.

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Blood tests are analyzed to estimate the GFR and the amount of creatinine present in the body [2]. Barr et al. (2004) showed that creatinine levels could also be measured in urine samples. This finding has resulted in its application to laboratories as one of the routine laboratory tests [3].

As far as the researchers are aware, no studies have been conducted about comparing creatinine levels from dried urine samples on filter paper with different temperatures, containers, and durations simultaneously.

The primary objective of this research was to identify the stability of creatinine levels from dried urine samples on filter paper with regards to different storage temperatures, containers, and durations to support an accurate method of alternative for the routine urine creatinine test. Specifically, this study aimed:

- To determine the effect of the following physical factors on the level of creatinine from the urine samples dried on filter paper:
  1. Durations
     - One week
     - One month
  2. Temperatures

- Room temperature (25℃)
- Refrigerator temperature (4℃)

3. Containers
   - Ziplock
   - Microwavable plastic
   - container

- To distinguish the physical variables that make the creatinine level of dried urine samples comparable to fresh samples.

- To determine which among the storage conditions will have the greatest effect on the creatinine level of dried urine samples.

This study primarily focused on the stability of creatinine levels of urine samples dried on filter paper considering different temperatures, containers, and durations. This study was limited to the examination of creatinine levels only, excluding the analysis of other substances found in urine. The researchers solely used the reagent strip method to determine the creatinine levels. Environmental conditions such as humidity, light exposure, atmospheric pressure, and others not mentioned were not covered.

## II. METHODOLOGY

### A. Research Design

Experimental design was used in this study. The researchers aimed to investigate the stability of creatinine levels of dried urine samples on filter paper with regards to different physical variables as a validation and further exploration of a recently published study of Tarik et al. (2019) on the feasibility of measuring sodium, potassium, and creatinine concentrations from urine samples dried on filter paper [4]. The physical variables served as the independent variables and were composed of different storage temperatures, conditions, and durations. Three (3) liquid urine samples were obtained from the researchers themselves residing in the National Capital Region (NCR). The samples were divided into control and experimental samples which were analyzed using Insight Urinalysis Reagent Strips for Microalbumin/Creatinine. The variables that showed the least difference in the creatinine levels between the control and experimental groups were identified and established the optimal conditions of storage and transportation for maintaining the stability of creatinine on the filter paper strips.

### B. Research Locale

The specimen was collected from researchers residing in NCR, for efficiency and convenience for the researchers as it is also within reach of the Pastrana Laboratory where the actual experiment was conducted. Data gathering was performed in the aforementioned laboratory located in Banga 1st, Plaridel Bulacan and was selected for its ability to accommodate the study.

### C. Sampling Procedure

The sample size was limited to three (3) participants due to the challenges brought about by the COVID-19 pandemic and was determined through a non-parametric, purposive sampling. To compensate for the small sample size, each specimen was triplicated to ensure the accuracy and validity of the results. An online form was deployed to screen the participants residing in NCR to ensure that they have no known history, abnormalities, and conditions (e.g., Diabetes, high cardiac output, burns, congestive heart failure, impaired kidney function, and hemorrhage) and do not consume any substance (e.g. Sulfamethoxazole/ trimethoprim, cephalosporins, fenofibrate, and acid blockers) that affects creatinine concentrations. This screening checklist was used to prevent outliers in the creatinine values. Candidates that gave their consent and met the inclusion criteria were asked to be tested for serum BUN and creatinine at the expense of the researchers for the validation of normal values prior to data collection.

### D. Data Collection

Three (3) urine samples were collected from three (3) participants aged 20 to 30 years old residing in NCR who have met the inclusion criteria, followed up by serum BUN and creatinine testing to validate the participants' normal kidney functions with no existing renal problems. Due to the pandemic's health risk, both the researchers and the study participants wore proper Personal Protective Equipment (PPE) such as face masks, face shields, gloves, and medical overalls that the researchers provided. Each participant was asked to answer a Health Declaration form prior to collection to ensure the safety of both parties involved in the study. Ethical clearance was obtained from the University of Santo Tomas Faculty of Pharmacy Research Ethics Committee, and written consent was asked from the participants involved in the study prior to urine collection.

The participants were orally instructed to collect their first voided urine in the morning and were provided with single 60 mL urine containers. Specimen collection was performed at ambient temperature (30°C) in the affiliated laboratory. The urine samples were immediately processed, stored, and disposed of within two (2) hours after use, following proper protocols.

### E. Sample Preparation

Upon collecting the specimens from the participants, exactly 0.5 mL of the urine sample was dropped onto the Grade 3 Whatman filter paper strip. Five (5) centimeters of the strip were drowned with urine, leaving the upper area for sample identification (2.5 cm) and diffusion capacity (2.5 cm). The strips were hung on the edge of a nonabsorbent thermocol sheet with the aid of adhesive tape, carefully not allowing the strips to touch each other, which will allow air-drying for two hours. Once completely dried, each of the urine strips was stored in different temperatures, containers, and durations. One (1) urine specimen was divided into a total of nine (9) filter paper strips, (8) eight filter paper strips that were stored in different conditions, and one (1) dried urine filter paper baseline. The eight (8) urine filter paper strips were divided and stored into two (2) types of containers, each with a desiccant, namely a microwaveable plastic container (Propylene) and ziplock (Polyethylene). Each of the two container groups was divided into four (4) urine strips and was further divided to be stored for one (1) week and one (1) month. Each of these two urine filter paper strips was stored to a fixed temperature of 25ºC and 4ºC, respectively. This process was repeated three (3) times for each sample for the validity and accuracy of the results. In order to have a point of comparison for the manipulated variables, two (2) baselines were used. One was the actual liquid urine sample, and the other was the urine dried on a filter paper strip that was processed and tested immediately for creatinine once dried. Considering the number of participants, 72 dried urine filter paper strips, three dried urine, and three liquid urine samples for the baseline were measured.

*Elution of Creatinine from Filter Paper Strips:*

The study done by Tarik et al. (2019) on the feasibility of measuring sodium, potassium, and creatinine concentrations from urine samples dried on filter paper was the basis of the sample analysis wherein the calibrators, urine controls, and samples prepared on the filter paper strips were eluted with deionized water. Eight (8) spots with a 6 mm diameter in each filter paper strip were punched out individually and placed in a 5 mL test tube. Deionized water measuring 350 µl was added to each test tube. The filled out test tubes underwent incubation with shaking at 250 rpm for 45 minutes at 37°C. The test tubes were centrifuged for 20 minutes at 3000 rpm, and 50 µl of supernatant from each test tube was taken for reagent strip analysis [4].

*Estimation of Creatinine Levels in Liquid and Dried Urine Samples:*

The estimation of creatinine levels present in both liquid and dried urine samples were done by reagent strip analysis using Insight Urinalysis Reagent Strips (Ref no. UC31-025) for Microalbumin/Creatinine. The principle of the reagent strips make use of the peroxidase-like activity of copper creatinine complex that catalyzes the reaction of diisopropyl benzene hydroperoxide and 3,3',5,5'-tetramethylbenzidine to produce a color range from orange to green to blue for the determination of creatinine concentration. This type of reagent strip measures two (2) parameters, namely creatinine and albumin levels, and is preferred for random testing, as it does not require 24-hour urine specimens. The results can be read visually after 60 seconds, producing three (3) semi-quantitative results of microalbumin, creatinine, and microalbumin/creatinine ratio in a single test. Estimation using this test provides a prediction of microalbuminuria, glomerular damage, and early kidney disease.

For the estimation of creatinine levels in the liquid urine and dried urine strip baselines, the reagent strip was immersed into the sample. The reagent areas were compared to the corresponding color blocks on the canister labeled after one minute. For the estimation of creatinine levels in the urine dried on the filter paper, the same process was done except that the supernatant obtained from the elution of the samples was used as a substitute for the liquid urine. To ensure the quality of the results, two urine controls were used for monitoring the accuracy and precision of the Insight Microalbumin and Creatinine Reagent Strip, specifically with the use of Insight Liquid Urine Chemistry Control (Ref no. U021-15). It is a liquid stable control prepared from simulated human urine with added chemicals, and constituents, using the routine procedure.

### F. Data Analysis

The statistical analysis was carried out using the software IBM SPSS which contains a library of machine learning algorithms such as the analysis of variance (ANOVA) method and paired sample statistics used to analyze the obtained creatinine levels.

*Liquid Urine Vs. Dried Urine Creatinine:*

A total of three (3) urine samples were analyzed in triplicates and were compared in both dried and liquid forms for the measurement of creatinine levels through paired sample statistics. Paired sample statistics were used to determine the mean difference between two sets of observations obtained from dried and liquid urine.

*Storage Stability of Urine:*

The three-way ANOVA was used to analyze the stability of creatinine levels with regards to the varying storage conditions, namely the container used, the temperature, and the time durations.

*Ideal Storage Condition*:

Paired sample statistics were used to determine the ideal storage conditions where the least variation in the creatinine levels of the dried urine stored in comparison to that of the baseline values. The same statistical analysis was done to determine the least ideal storage conditions where the greatest variation in the creatinine from the baseline was recorded.

## III. Results

The stability of creatinine levels of urine dried on filter paper under different storage durations (one week and one month), temperatures (room temperature of 25°C and refrigerator temperature of 4°C), and containers (ziplock and microwavable plastic container) were determined. Liquid and dried urine baseline creatinine levels both reflected with the same statistical values (n=72, mean = 116.67 mg/dL, SD = 62.799, Std error mean = 7.401). Paired sample statistics were used to determine the correlation between the liquid and dried urine baseline values. The creatinine levels of the liquid and dried urine baseline values revealed a standard error of the difference, which is 0. Thus, the correlation and t-test values cannot be computed.

Table.1. Comparison of Creatinine Levels from Dried and Liquid Urine Samples.

| | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| Baseline (Liquid Urine) | 116.67[a] | 72 | 62.799 | 7.401 |
| Baseline (Dried Urine) | 116.67[a] | 72 | 62.799 | 7.401 |

a. The correlation and t cannot be computed because the standard error of the difference is 0.

A three-way analysis of variance was conducted to compare the main effects of duration, temperature, and container on the amount of creatinine of three (3) urine samples subjected to three (3) trials. The coefficient of determination revealed an R-squared value of 0.073, indicating that the independent variables (duration, temperature, and container) did not account for the changes in creatinine levels of dried urine samples and the variation of creatinine values from the baseline. Results revealed no significant difference (F-value = 0.627, p-value > 0.05) between the independent variables and the creatinine values from the baseline. In addition, there is no significant two-way interaction between duration and temperature (F-value = 0.157, p-value > 0.05), between duration and container (F-value = 1.557, p-value > 0.05), and between container and temperature (F-value = 0.627, p-value > 0.05).

Table.2. Summary of Three-way ANOVA of Creatinine Values from Dried Urine Samples Stored under Different Conditions.

**Tests of Between-Subjects Effects**

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 4444.444[a] | 7 | 634.921 | .717 | .658 |
| Intercept | 8888.889 | 1 | 8888.889 | 10.039 | .002 |
| Duration | 555.556 | 1 | 555.556 | .627 | .431 |
| Temperature | 1250.000 | 1 | 1250.000 | 1.412 | .239 |
| Container | 1250.000 | 1 | 1250.000 | 1.412 | .239 |
| Duration & Container | 138.889 | 1 | 138.889 | .157 | .693 |
| Temperature & Container | 555.556 | 1 | 555.556 | .627 | .431 |
| Duration & Temperature & Container | 555.556 | 1 | 555.556 | .627 | .431 |
| Error | 56666.667 | 64 | 885.417 | | |
| Total | 70000.000 | 7 | | | |

Dependent Variable: Change in Creatinine Level (Difference between baseline and final amount creatinine level)
R Squared = .073

### A. Durations (One-Week Vs. One Month)

The creatinine values in dried urine samples were observed to be stable during the storage duration of one month at both 25°C and 4°C in a ziplock and microwavable plastic containers compared to the baseline values of liquid and dried urine samples. The calculated t-value was t(70) = -0.801 (p > 0.05), which indicates that there is no significant difference in the creatinine levels of dried urine samples stored for one week and one month**.**

The creatinine levels of dried urine samples stored for one month showed a larger difference in the mean difference and standard deviation (mean=13.89 mg/dL, std. dev.=32.974 mg/dL) compared to the ones stored for one week (mean=8.33 mg/dL, std. dev.=25.355 mg/dL).

Table.3. Test of Difference on Creatinine Levels of Urine Dried on Filter Papers After One Week and One-Month Storage.

| Duration | N | Mean | Std. Deviation | Std. Error Mean | Difference | t-test | df | p-value |
|---|---|---|---|---|---|---|---|---|
| One week | 36 | 8.33 | 25.355 | 4.226 | -5.56 | -0.801 | 70 | 0.426 |
| One month | 36 | 13.89 | 32.974 | 5.496 | | | | |

### *B. Temperatures (4°C Vs. 25°C)*

The test of difference for comparing creatinine levels of dried urine samples stored under 25°C and 4°C revealed a t-value of 1.209 ($p > 0.05$), suggesting no significant difference in creatinine levels when stored under both temperatures. Creatinine levels in dried urine samples stored at 25°C showed a larger mean difference and deviation (mean=15.28 mg/dL, std. dev.=33.422 mg/dL) than the ones stored under 4°C (mean=6.94 mg/dL, std. dev.=24.357 mg/dL).

Table.4. Test of Difference on Creatinine Levels of Urine Dried on Filter Papers under Room (25°C) and Refrigerated (4°C) Temperatures.

| Temperature | N | Mean | Std. Deviation | Std. Error Mean | Difference | t-test | df | p-value |
|---|---|---|---|---|---|---|---|---|
| 25°C | 36 | 15.28 | 33.422 | 5.570 | 8.34 | 1.209 | 70 | 0.231 |
| 4°C | 36 | 6.94 | 24.357 | 4.059 | | | | |

### *C. Containers (Microwavable Plastic Container Vs. Ziplock*

The test of difference for comparing creatinine levels of dried urine samples stored in ziplock and microwavable plastic containers showed a t-value was 1.209 ($p > 0.05$), which suggests that there is no significant difference in the creatinine levels stored in both containers.

Creatinine levels of dried urine samples stored in ziplock bags showed a slightly larger mean difference and deviation (mean=15.28 mg/dL, std. dev. = 5.570 mg/dL) compared to the ones stored in microwavable plastic containers (mean=6.94 mg/dL, std. dev.=4.059 mg/dL).

Table.5. Test of Difference on Creatinine Levels of Urine Dried on Filter Papers Stored in Ziplock and Microwavable Plastic Containers.

| Container | N | Mean | Std. Deviation | Std. Error Mean | Difference | t-test | df | p-value |
|---|---|---|---|---|---|---|---|---|
| Ziplock | 36 | 15.28 | 33.422 | 5.570 | 8.34 | 1.209 | 70 | 0.231 |
| Plastic Microwavable container | 36 | 6.94 | 24.357 | 4.059 | | | | |

Table.6. Test of Difference on the Creatinine Levels Placed in Ziplock and Microwavable Plastic Container Under 25°C and 4°C for One Week and One Month.

| Duration | Temperature | Container | N | Mean | Std. Deviation | Difference | t-test | df | p-value |
|---|---|---|---|---|---|---|---|---|---|
| One week | 25°C | Ziplock | 9 | 16.67 | 35.355 | 5.56 | 0.343 | 16 | 0.736 |
| | | Plastic microwavable container | 9 | 11.11 | 33.333 | | | | |
| | 4°C | Ziplock | 9 | 5.56 | 16.667 | 5.56 | 1.000 | 16 | 0.332 |
| | | Plastic microwavable container | 9 | .00 | .000 | | | | |
| One month | 25°C | Ziplock | 9 | 16.67 | 35.355 | 0.00 | 0.000 | 16 | 1.000 |
| | | Plastic microwavable container | 9 | 16.67 | 35.355 | | | | |

### *D. Optimal Storage Condition*

Test of difference on the creatinine levels revealed that there is no significant difference in the change of creatinine when the urine dried on filter papers are placed in ziplocks and microwavable plastic containers for one week at 25ºC [t-value (16) = 0.343, $p > 0.05$] and 4°C [t-value (16) = 1.00, $p > 0.05$]. Storage for one month in ziplocks and microwavable plastic containers also showed no significant difference in the change of creatinine levels under 25ºC [t-value (16) = 0.00, $p > 0.05$] and 4°C [t-value (16) = 1.512, $p > 0.05$].

Despite showing no significant difference between creatinine levels stored in ziplocks and microwavable plastic containers for one week and one month at 25ºC and 4ºC, creatinine concentrations in different physical variables still show some deviation. The combination of storage conditions involving the microwavable plastic container stored at 4ºC in both durations of one week and one month showed a mean difference of 0.0 mg/dL and a standard deviation of 0.0. This signifies that the creatinine levels of dried urine samples kept in a microwavable plastic container at 4ºC remained constant for one week and one month.

Storage of one month under 4°C in ziplocks showed a larger mean difference of 22.22 mg/dL than the 25ºC under the same duration and container, 16.67 mg/dL.

On the other hand, one week's storage at 25ºC in ziplocks showed a mean difference of 16.67 mg/dL and is greater than the ones in ziplocks at 4°C, which is 5.56 mg/dL.

One month's storage of urine dried on filter paper strips at 4°C in ziplocks exhibited the greatest variation of creatinine levels and largest mean value of 22.22 mg/dL.

## IV. Discussion

Measurement of creatinine levels is usually performed using 24-hour urine samples. However, collecting this type of sample is often time-consuming, more expensive, and prompts inconvenience and difficulty among the children, elderly, and disabled patients. To provide ease in transportation and storage, alternative methods for measuring creatinine levels have been developed using the filter paper-based method or urine dried on filter paper strips with either 24-hour or random urine samples. There are studies that have proven the stability of creatinine levels when measured in liquid and dried urine samples; however, no current study regarding the stability of creatinine levels of dried urine samples when subjected to different physical factors. Thus, this study was conducted to determine the optimal conditions to stabilize the creatinine levels of dried urine samples.

In this study, creatinine levels of urine dried on filter paper strips were assessed. Paired sample analysis showed a zero mean difference between the liquid and dried urine creatinine levels suggesting that dried urine samples can reliably be employed for measuring the analyte. The stability of creatinine levels of urine dried on filter paper under different storage durations, temperatures, and containers were determined. A three-way analysis of variance revealed an R-squared value of 0.073, indicating that the independent variables (duration, temperature, and container) did not account for the changes in creatinine levels of dried urine samples and the variation of creatinine values from the baseline. Therefore, the null hypothesis must be rejected. With this, the results confirm that dried urine samples can be used to measure creatinine and are virtually unaffected by the storage time, temperature, and container.

The greater stability of creatinine values in samples stored in microwavable plastic containers can be attributed to the fact that its main component, polypropylene, is highly resistant to chemicals and organic solvents compared to polyethylene in ziplocks (Hinsley, 2016). Since urine is naturally acidic, polypropylene is a more suitable container for storing urine samples due to its chemically inert and non-toxic nature. This is also the reason why most specimen containers are made out of the said material [5].

Liquid urine specimens are usually collected and stored in specimen containers then placed in resealable plastic bags such as ziplock bags. Urine dried on filter paper was discovered to stabilize different analytes for easy handling and transportation. According to a study done by Tarik et al. (2019), creatinine levels in 134 urine dried on filter paper samples stored for one year in ziplock under 4°C exhibited a large mean difference (77.81 mg/dL) and deviation (45.02 mg/dL) in comparison with the liquid baseline values, using a Bland–Altman plot for agreement analysis. This result was consistent with another study where creatinine levels were determined in 49 paired liquid and dried urine samples using the same statistical analysis [4].

The common denominator of the two largest mean values from the test of difference on creatinine levels is the use of ziplock. The least stable creatinine values were observed in the one-month storage of dried urine samples in ziplock bags kept at 4°C, demonstrating large differences noted in the triplicated samples and was accordant with the variables that exhibited significant differences based on the other studies.

## V. Conclusion

By subjecting the samples to various physical factors, the results regarding the use of fresh liquid and dried urine samples showed no significant difference on the stability of creatinine levels. Therefore, any combination of the physical factors can be used for storage and transport of dried urine samples. The relationships of storage duration (one week and one month), container (microwavable plastic container and ziplock), and temperature (room temperature of 24°C and refrigerator temperature of 4°C) of the dried urine samples showed no significant statistical differences. Comparison between individual conditions revealed that a temperature of 4°C has a greater stability than 24°C, microwavable plastic containers are more stable than ziplocks, and between the two storage durations, one week has greater stability in comparison with one month. The combination of storage in plastic tupperware for one week at 4°C exhibited the optimal storage condition for dried urine samples while the greatest difference in creatinine levels was observed in storing the samples in ziplock for one month at 4°C. Conclusively, it was proven that all physical factors have no significant effects on the creatinine levels of dried urine samples.

For further studies, the researchers recommend the use of more dried urine samples obtained from a broader range of participants to better represent the population, and to test the stability of both normal and abnormal creatinine values. A quantitative creatinine assay for measuring the creatinine levels can also be used to eliminate subjective biases or interferences from using reagent strips, thus increasing the specificity of the data. The inclusion of more variables in accordance with the proposed objectives is also recommended to further identify conditions that may affect the stability of creatinine levels.

*Ethical Considerations*:

This study has been granted ethical approval by the University of Santo Tomas Faculty of Pharmacy Research Ethics Committee and is provided with the study protocol code FOP-REC-2021-01-038.

*Conflict of Interest:*

The authors of this study declare that the research was conducted without any conflict of interest.

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