# Correlation between the creatinine clearance in the urine collected during 24 hours and 12 hours

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## ABSTRACT

**Introduction:** Creatinine concentration in plasma has been used to evaluate renal function. However, the endogenous creatinine clearance (CrCl) is more sensitive to this goal. **Objective:** Correlate the CrCl calculated from urinary collects of 12 h and 24 h. **Methods:** Ninety five volunteers (34-64 y) collected the urine for 24 h into two bottles: night, from 7 am to 7 pm and day, from 6 am to 7 pm. A fasting blood sample was used to measure plasma creatinine. Correlation between variables was determined by Pearson method (r) and the agreement between night and 24 h CrCl was determined by the Bland-Altman plot. **Results:** Urines of 4 individuals were discarded because of collect errors. In the final sample (n = 91; 42 males), hypertension was found in 23 and diabetic in 5. The CrCl (mL/min/1.73 m$^2$) was slightly lower in females in the night (77.8 ± 22.7 versus 88.4 ± 23.6; p < 0.05) and similar in males (91.2 ± 22.9 *versus* 97.3 ± 30.9; p > 0.05). Strong correlations were observed between the CrCl calculated from the night and day urines and the 24 h (r = 0.85 and 0.83; respectively). Agreement between the CrCl calculated from night or day urine and the 24 h urine was observed, respectively, to 85 and 83 individuals. **Conclusion:** The 12 h urine, mainly obtained at night, gives CrCl values similar to those obtained in the 24 h collect. Since urine collect is easier to outpatients at night, this period should be chosen in the clinical evaluation of the glomerular filtration rate.

**Keywords:** creatinine, glomerular filtration rate, kidney function tests, kidney failure chronic.

## INTRODUCTION

The increase in the incidence of chronic renal disease is a serious problem of public health worldwide.[1] In Brazil, the number of patients on renal dialysis programs more than doubled in the past decade, and the demand for renal replacement therapy increased at rates close to 10% a year.[2] Renal diseases, when not diagnosed and treated early, can evolve to chronic renal failure, determining incapacity for work or premature death.[3,4] Thus, early identification of renal impairment is fundamental for adopting therapeutic strategies for renal parenchyma protection, aiming at preserving renal function.

In daily clinical practice, qualitative assessment of the kidney excretory capacity is usually performed by measuring serum creatinine concentration. However, several studies have shown that, although having that marker within the normal range, a significant amount of individuals already have impaired renal function, which can be detected by more sensitive methods, such as serum creatinine clearance (CrCl).[5,6] Thus, CrCl is a safer and more accurate method to assess renal function than the isolated measurement of serum creatinine.[3,7]

Measuring CrCl requires urine collection for a predetermined period of time. The reference values of that measurement have been established for 24-hour collections,[8] which is an important limitation for the use of CrCl in non-hospitalized patients, especially in the earliest phases of renal disease, when individuals still work. Aiming at overcoming those difficulties, formulae for calculating CrCl that do not require urine collection, since they are based on

measuring serum creatinine concentration, body weight, age, and sex, have been established.[3,7,9] A drawback in using such formulae, however, is their low correlation with direct glomerular filtration measures and their low sensitivity for detecting earlier stages of renal dysfunction.[10,11] That fact becomes even more important due to the observation that preserving renal function in chronic diseases, such as diabetes and arterial hypertension, requires the implementation of early measures of renal protection.[3,10]

The difficulty in collecting all urine produced during 24 hours is one of the most important limitations for correctly obtaining CrCl in the clinical context and also in epidemiological research. In a previous study, we showed the good applicability of 12-hour urine collection during the night for assessing sodium consumption.[12] In this study, our major objective was to assess the degree of correlation between CrCl measured in urine collected for 12 hours and that measured in urine collected for 24 hours.

## METHODS

This study was carried out with a convenience sample comprising individuals of different educational and socioeconomic levels. The project was announced among the servers of the Center of Health Sciences (CCS) of the Federal University of the Espírito Santo State (UFES) and participants of a community program of physical activity maintained by the Vitória City Hall, close to the CCS. The project was verbally announced in those two places for small groups, with the information that participants would undergo routine exams for determining cardiovascular risk, such as blood pressure measurement, electrocardiography, and blood tests. A total of 114 individuals completed the pre-adherence form, and 95 individuals (aged from 34 to 64 years) attended the preparation meeting (groups of six to ten) to receive detailed instructions about the nature of the project and procedures for urine collection. At the end of the meeting, each participant received the material for urine collection, in addition to written instructions about the procedure. The project was approved by the Committee on Ethics and Research of the CSS of the UFES (n.o 041/2006). All participants provided written informed consent.

### Urine collection

Urine collection was programmed for a 24-hour period and should be performed in two bottles labeled as follows: day urine (from 7AM to 7PM) and night urine (from 7PM to 7AM). Each participant received

two previously-labeled, sterile bottles with a wide-opening, and a form with instructions and blank space for taking notes about the effective starting and ending hours of collection for each period. The individuals were instructed to maintain their usual diet during the day and to fast after 8 PM. The day urine bottle should be packed in a plastic bag and kept in the refrigerator. After the last collection of the morning, the participants should go to the university-affiliated hospital to deliver the urine, draw blood, provide sociodemographic data, and undergo clinical exams (weight, height, blood pressure, and rest electrocardiography).

Upon arrival at the place of the exams, the notes about the urine collection hours were checked. Four participants were excluded from the analysis because of collection errors (error with a tolerance of up to 1 hour for each bottle) or 24-hour urine volume lower than 500 mL. Urine volume was measured by use of a graduated cylinder with 10 mL accuracy. Aliquots of 2 mL were collected in sterile tubes and sent for measuring sodium, potassium, urea, and creatinine. Total 24-hour excretion was obtained by adding the two periods. Blood was collected during fasting by venipuncture of the forearm, and urea and creatinine concentrations were considered stable for a 24-hour period. All measurements were performed at a single laboratory by using commercial kits.

Blood pressure was measured on the forearm during fasting, with the individual seated and after a rest of at least 5 minutes, by using an oscillometric device (Omron 765 CP IntelliSense). Individuals with blood pressure values ≥ 140/90 mm Hg or using anti-hypertensive medication, including diuretics, were classified as hypertensive. Those with fasting glycemia ≥ 126 mg/dL or using insulin or oral antidiabetic drugs were classified as diabetic. Demographic data (age, sex, educational level) and life habits (smoking, alcohol consumption, practice of physical activity) were provided in an interview. The intensity of obesity was determined by measuring body mass index (BMI). Body surface was calculated by use of the Dubois formula,[13] and CrCl was corrected to 1.73m².

Data were expressed as mean ± standard deviation or as proportions. The Kolmogorov-Smirnov test was used to test the normality of the distribution of continuous variables. Means were compared by use of the Student t test, and proportions were compared by use of the X2 test. The degree of association between variables was determined by use of Pearson correlation coefficient (r), and the degree of agreement between CrCl in 12-hour and 24-hour urine samples was assessed by use of the Bland-Altman plot.[14] The number of

individuals (N) was established for detecting r > 0.80, with a beta error lower than 5%. All statistical calculations were performed in the SPSS program, version 13.1 (Chicago, IL, USA). The statistical significance level adopted was p < 0.05.

## RESULTS

Because four participants were excluded, data presented refer to 91 participants (42 men and 49 women). The sample included individuals with different educational levels (superior, 11 participants; middle level, 31; and elementary level or lower, 49) and socioeconomic levels (classes A + B, 25; class C, 61; classes D + E = 11 individuals). The major anthropometric,

clinical, and laboratory characteristics of the sample are shown in Table 1. The systolic pressure values and the serum concentrations of creatinine and uric acid were lower (p < 0.05), and HDL-cholesterol was higher (p > 0.05) in women. Serum creatinine was equal to or lower than 1.2 mg/dL in all individuals. The prevalences of hypertension, diabetes, and smoking were, respectively, 25%, 5.5% and 16%, with no difference between sexes. Of the five diabetic individuals, two had glycemia ≥ 200 mg/dL (200 mg/dL and 318 mg/dL) and 24-hour urine volumes of 0.7 L and 4.09 L, respectively.

The characteristics of day and night urine are shown in Table 2. Urine volume and, consequently, urine

| Table 1 | GENERAL CHARACTERISTICS OF THE SAMPLE | | |
|---|---|---|---|
| Variable | Men (n = 42) | Women (n = 49) | All (n = 91) |
| Age (years) | 48 ± 8.2 | 47 ± 7.5 | 47 ± 7.8 |
| BMI (kg/m$^2$) | 26.5 ± 3.6 | 27.2 ± 4.4 | 26.9 ± 4.1 |
| SBP (mm Hg) | 123 ± 17 | 115 ± 14* | 119 ± 16 |
| DBP (mm Hg) | 79 ± 12 | 76 ± 8 | 77 ± 9 |
| Glucose (mg/dL) | 101 ± 36 | 93 ± 19 | 97 ± 28 |
| Total cholesterol (mg/dL) | 189 ± 39 | 202 ± 41 | 196 ± 40 |
| HDL-cholesterol (mg/dL) | 41.2 ± 11.4 | 46.6 ± 9.2* | 44.1 ± 10.6 |
| Triglycerides (mg/dL) | 143 ± 95 | 117 ± 102 | 129 ± 99 |
| Creatinine (mg/dL) | 1.0 ± 0.09 | 0.90 ± 0.10* | 0.95 ± 0.10 |
| Uric acid (mg/dL) | 4.7 ± 1.0 | 3.6 ± 0.9* | 4.1 ± 1.0 |

Data correspond to mean ± standard deviation.
N: number of individuals; BMI: body mass index; SBP: systolic blood pressure
DBP: diastolic blood pressure (* p < 0.05; man *versus* woman).

| Table 2 | CHARACTERISTICS OF URINE COLLECTED IN THE DAY AND NIGHT PERIODS | | | | | |
|---|---|---|---|---|---|---|
| | Men | | | Women | | |
| | Day | Night | 24 hours | Day | Noturno | 24 hours |
| Volume (L) | 1.07 ± 0.54 | 1.08 ± 0.51 | 2.15 ± 0.97 | 1.11 ± 0.55 | 1.11 ± 0.48 | 2.22 ± 0.92 |
| Urine flow (mL/min) | 1.48 ± 60.75 | 1.50 ± 0.71 | 1.49 ± 0.67 | 1.54 ± 0.76 | 1.54 ± 0.67 | 1.54 ± 0.64 |
| Na$^+$ (mEq) | 117.0 ± 42.8 | 109.3 ± 50.8 | 226.3 ± 78.0 | 113.7 ± 43.4* | 91.7 ± 35.0 | 205.3 ± 63.2 |
| K$^+$ (mEq) | 36.8 ± 13.6* | 23.5 ± 8.6 | 60.2 ± 18.9 | 34.8 ± 11.3* | 21.3 ± 11.2 | 56.1 ± 17.9 |
| Urea (g) | 10.8 ± 3.5 | 10.7 ± 3.3 | 21.4 ± 6.0# | 10.0 ± 3.9 | 8.7 ± 2.9 | 18.7 ± 6.0 |
| Creatinine (mg) | 763.4 ± 245.8# | 718.8 ± 180.5# | 1.482.2 ± 368.6# | 541.6 ± 159.9* | 481.5 ± 164.5 | 1.023.1 ± 292.3 |
| CrCl (mL/min/1.73 m$^2$) | 97.3 ± 30.8 | 91.2 ± 22.9# | 94.3 ± 23.4# | 88.4 ± 23.6* | 77.8 ± 22.7 | 83.1 ± 19.9 |

CrCl: serum creatinine clearance. Urine of the day was collected from 7AM to 7PM; of the night, from 7PM to 7AM of the next day. Data were shown as mean ± standard deviation. (*) p < 0.05; day versus night in the same sex. (#) p < 0.05; men *versus* women for the same period of collection.

flow were similar in men and women, with no significant difference between the collection periods. The mean sodium excretion was 215 mEq over 24 hours, corresponding to a 4.9-g sodium intake per day (equivalent to 12.5 g of NaCl). Sodium intake tends to be greater in men, but the difference disappears after correcting for body weight (3.09 ± 1.20 mEq/kg and 3.30 ± 0.86 mEq/kg, in men and women, respectively; p = 0.80). Total sodium excretion was greater during the day, but with statistical significance only for women. Potassium was more excreted during the day for both men and women.

The 24-hour urea and creatinine excretion values were higher in men. Regarding creatinine, that difference was reduced when the values were normalized for body weight (19.7 ± 4.2 mg/kg in men and 15.0 ± 3.3 mg/kg in women; p < 0.01). The relative urea excretion, however, was similar in men and women (0.14 g/kg and 0.15 g/kg, respectively) after correcting for body weight.

The 24-hour CrCl was higher in men, and a small difference in the day and night periods was observed only in women. According to the limits of normal[8], which depend on age and sex (from 34 to 39 years, 97 and 103; from 40 to 49 years, 88 and 81; from 50 to 59 years, 81 and 74; and from 60 to 64 years, 72 and 63 mL/min/1.73m$^2$, for men and women, respectively), 13 men and 23 women had CrCl values below the limits of normal. However, when applying the most clinically used cutoff point for identifying renal dysfunction 3 (60 mL/

min/1.73m$^2$), only four men and one woman were below that limit.

The correlation between nocturnal CrCl and 24-hour CrCl is shown in Figure 1, and a strong association is observed between those variables both in the total sample (r = 0.848; p < 0.001) and after stratifying for sex (r = 0.825; p < 0.001; r = 0.854, p < 0.001, for men and women, respectively). Assuming linearity between both measures, the 24-hour CrCl can be estimated by the equation:

$$CrCl\ 24h = 21.3 + 0.80 \times CrCl\ 12h\text{-night}$$

The 95% confidence interval for the linear coefficient was 12.2 - 30.4; for the angular coefficient, it was 0.69 - 0.90. Similar results were observed for CrCl24h and CrCl12h-day (r = 0.83; CrCl24h = 21.5 + 0.72 x CrCl12h-day). A significant, but moderate, correlation (r = 0.448; p < 0.05) was observed between CrCl24h and that calculated through the Cockcroft-Gault formula corrected for sex, as shown in Figure 2. The correlation between CrCl24h and CrCl estimated by the MDRD formula was not significant (r < 0.20; p > 0.05).

The degree of agreement between the CrCl values in 24 hour urine and in 12 hour-night urine is shown in Figure 3. Of the 91 urines assessed, 85 were within the limits of agreement (± 2 standard deviations). Three of the six measures outside this range

**Figure 1.** Correlation between CrCl measured by use of urine collected during the night and CrCl calculated by use of 24-hour urine collection. The graph shows the regression line and the 95% confidence interval of the regression. R = Pearson correlation coefficient.
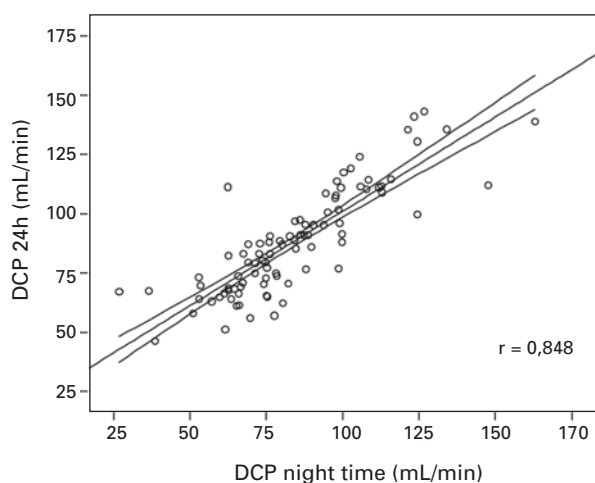
**Figure 2.** Correlation between CrCl calculated by use of the Cockcroft-Gault formula adjusted for sex and the CrCl calculated by use of 24-hour urine collection. The graph shows the regression line and the 95% confidence interval of the regression. R = Pearson correlation coefficient.
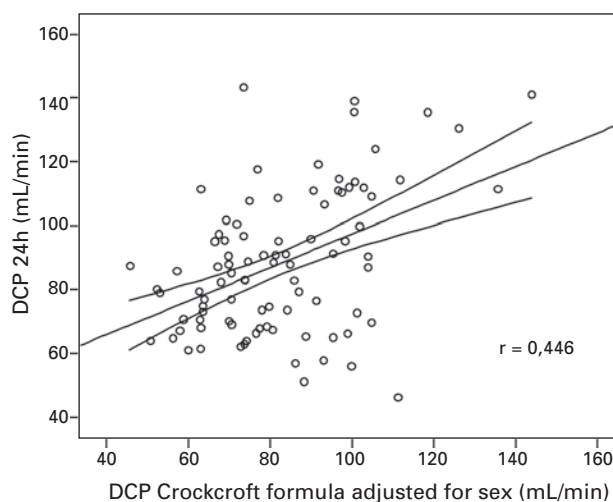
**Figure 3.** Bland-Altman plot for determining the degree of agreement between CrCl measured by use of 24-hour urine collection (A) and night urine collection (B). Note that only six individuals were outside the limits of agreement between both methods (dotted lines).
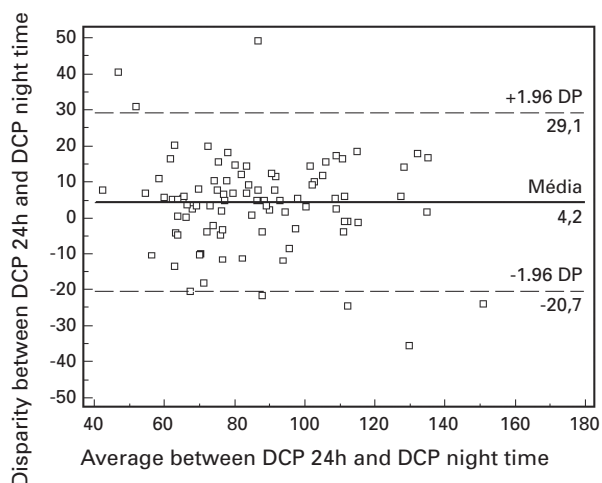


lye greatly away from the extremes of agreement (± 2 standard deviations). According to the Bland-Altman plot, no evidence of bias is observed when estimating the 24-hour CrCl based on urine collected only during the night, since individual data are randomly distributed below and above the central middle line. Regarding the urine collected during the day, agreement was slightly lower (83 individuals within the limits of agreement).

## DISCUSSION

The appearance of chronic renal dysfunction is a signal of poor prognosis in several highly prevalent diseases, such as arterial hypertension and diabetes. Renal impairment, regardless of its cause, should always be detected in its initial stages, when renal disease is still asymptomatic. In that phase, serum creatinine concentration is usually within the limits of normal. Based on that, the National Kidney Foundation (USA) has established practical rules, in which glomerular filtration rate plays an important role, for assessing renal function in the clinical context.[15] Based on serum creatinine and other easily-obtained indicators, such as gender, weight, and height, some formulae have been developed for estimating CrCl. The most accepted formulae were those developed by Cockcroft and Gault9 and by the MDRD study.[16] Calculating CrCl by using formulae, however, has some restrictions. The accuracy of the measure is not the same in some subgroups. In blacks, for example, the Cockcroft-Gault formula should be applied with corrections. The validation of

that correction factor, however, was performed only for Afro-Americans (USA),[17] and, so far, its applicability to other afrodescendent populations is unknown, and so is its validity for autochthonous African populations. There is also evidence that, for obese individuals, the Cockcroft-Gault formula overestimates the actual value of CrCl.[18] Another limiting factor is that studies on large samples have shown only a moderate correlation between CrCl estimated through formulae and that measured directly with different laboratory methods. That fact has been confirmed in our study, in which a correlation coefficient of only 0.48 was observed between CrCl measured in the 24-hour urine collection and that calculated by use of the Cockcroft-Gault formula. That value is close to the value found in more robust studies.[19] Therefore, even considering the easy application of formulae for estimating CrCl, the overlapping of two methods is only partial, and the probability of false positive and false negative results is high. In addition, the currently most used Cockcroft-Gault and MDRD formulae have been derived from populations with a high prevalence of renal dysfunction, and, thus, they do not reflect the situation of the general population.

Another factor to be considered is that urine collection in a predetermined time also plays an important role in verifying other renal function parameters, such as the quantitative determination of protein loss or the total excretion of nitrogen substances, in addition to estimating glomerular filtration. Another advantage of urine collection in a predetermined time is the possibility of estimating other parameters, such as sodium and potassium intake, which are highly useful for managing certain diseases, such as arterial hypertension, heart failure, and renal diseases in general. Therefore, timed urine collection allows the acquisition of data of high diagnostic and prognostic importance, in addition to calculating CrCl.

The direct measurement of CrCl requires, however, total urine collection within a predetermined period of time. Incomplete emptying of urinary bladder and errors in starting and ending times of collection jeopardize the measures of urine flow. The reference values in the literature were fixed for 24-hour urine collections.[8] For many patients, collecting urine during such a long period of time has inconveniences, particularly carrying out the procedure away from home, such as at the workplace. This probably contributes to errors in measuring CrCl, which can reach up to 30% of the assessments of non-hospitalized patients.[20,21] In addition, urine collected for long periods of time, if not properly stored, can undergo

modifications in creatinine concentration due to the amount and quality of the bacteria contaminating the material collected. Thus, urine collection for a shorter period and also at a more convenient time for the patient, preferably coinciding with the patient being at home, can represent an important alternative for obtaining more accurate indicators of renal function.

In a previous population-based study, our research team had already collected urine for 12 hours at night to assess salt intake in the city of Vitória, in the State of Espírito Santo.[12] In that study, urine was correctly collected, without great difficulties for most individuals. Thus, the validation of that time period of collection for assessing CrCl is of practical interest for use in both non-hospitalized patients and epidemiological research.

Our data have shown a strong correlation between CrCl measurements in urine collected for 12 hours at night and those in urine collected for 24 hours. This study was conducted with volunteers, most of whom worked at the university, which could represent a greater interest in correctly collecting urine. This could not happen if measures were taken in non-hospitalized patients. The demographic characteristics of the sample, however, indicate participants were diverse in terms of age, culture (assessed through educational level), and economic level. Even being a convenience sample, those characteristics are similar to those of non-hospitalized patients seeking for health care. The prevalence of hypertensive and diabetic patients in the sample is similar to that in the general population; two subgroups for which a more accurate assessment of renal function has great clinical relevance.[3-5] Instructions on urine collection were especially emphasized. Each participant was given oral and written instructions, including one form in which the exact times of starting and ending urine collection in each period should be written down. The small number of errors in collection time indicates good acceptance and quick learning of the method. It is worth noting that all individuals had normal creatinine levels ($\leq$ 1.2 mg/dL), but four men and one woman already had CrCl lower than the reference values (60 mL/min/1.73m²). These data emphasize the importance of the direct calculation of CrCl for assessing renal function.

Some findings of the study are worthy of note. A slight decrease in CrCl was observed during the night (6% in men and 12% in women), coinciding with the reduction in glomerular filtration during sleep.[8] It is worth emphasizing, however, that greater circadian oscillations can be found in patients with renal disease, heart disease, or even in the elderly, whose redistribution of body fluids is strongly influenced by body position.[22] In our study, the individuals had neither edema nor advanced stage diseases, representing individuals usually cared for on an outpatient clinic regimen. However, we do not have an explanation for the difference found between men and women. Even with that physiological variation, the linearity between the 12-hour and 24-hour measures showed a strong correlation (r = 0.848; Figure 1). Thus, small errors resulting from physiological oscillations of CrCl do not seem to be an important limiting factor for the use of 12-hour urine collection. Potassium excretion, however, seems to obey a rhythm with greater circadian oscillation, since night excretion corresponded to less than 40% of the total potassium excretion over 24 hours. The finer regulation of serum potassium concentration may be responsible for that circadian variation, and the greater potassium urine excretion coincides with the period of greater potassium intake. This fact has not been observed for sodium.

The possibility of using shorter timed urine collections to estimate CrCl has already been tested in several previous studies, most of which, however, were conducted at a hospital or in patients with renal failure.[23,24] In a study carried out at a hospital, Markantonis and Agathokleous-Kioupaki[25] have reported that CrCl data from 8-hour urine collection performed from midnight to 8 AM were similar to those measured in 24-hour urine collection.

Finally, it is worth noting some limitations of this study. First, this study was conducted with a convenience sample, in which more than half of the individuals worked at the university, being, thus, probably more motivated to collect urine correctly. The small group meetings have also made correct urine collection easier. Second, the urine volumes were greater than those of other studies in the general population.[12] The fact that the individuals knew they were participating in the study may have made them drink more water. However, total creatinine excretion corrected for weight was within the limits of normal[8] (14.4 to 33.6 mg/kg in men and 10.8 to 25.2 mg/kg in women) for most individuals (except for five men and four women). Thus, one can conclude that 12-hour urine collection during the night is an adequate alternative

to 24-hour urine collection for determining CrCl. Shorter urine collection periods, when correctly timed, can also determine correct CrCl measures. However, further studies are required to determine whether collection during that period is also valid for other parameters of renal function assessment, such as 24-hour protein loss. Such studies should be conducted in populations with nephropathies. Urine collection for a shorter and more convenient period for the patient can reduce errors of urine collection, and, thus, increase the diagnostic accuracy of the test.

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