Using physical and chemical examinations of 24-hour and random urine samples to evaluate the urine component for a 21-year-old non-athlete male

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Abstract

Urinalysis is one of the effective measurements to evaluate the health status of individuals. A series of physical and chemical examinations, including urine volume, color, odor, pH, titratable acidity, specific gravity, microchemistry screening test, as well as the urinary calcium and creatinine, are commonly applied in the clinical diagnosis to assess the patients' physiological condition and kidney function. The purpose of this experiment was to evaluate the urine components of the subject by several measurements mentioned above. In this experiment, 24-hour and random urine samples of a 21-year-old non-athlete male were used to conduct these measurements. The results showed that most of the values are within the normal range, while values of titratable acidity and creatinine levels are lower than expected and need further check and analysis. The experiment demonstrated how different urinalysis measurements are applied to detect the components of urine, which could also be considered as early screening and diagnosis for certain diseases.

Introduction

Physical examinations of urine include urine volume, color, and odor. Urine volume is an important indicator in clinical treatment. It is strictly associated with solute intake among patients with hyponatremia, which could be a guidance for doctors to adjust the water intake and medicine use for patients in their treatment.[1] Study has found that residual urine volume is related to the mortality rate of patients on dialysis, which could be applied as a prediction for

patient survival.[2] For healthy individuals, 24-hour urine volume is an essential index to calculate the total substances in our urine, and the normal range is 800 - 2000 milliliters per day.[3] Urine color provides clues for clinical diagnosis, and could be affected by drug, food intake, and hydration status. Normal urine color ranges from light yellow to deep amber, which is typically clear and transparent.[4] Urine odor of healthy individuals is relatively mild, while unusual odor for certain populations is due to diseases, drugs, and diets.[5] In this experiment, the subject is in health condition and is expected to excrete 800 - 2000 mL urine in 24 hours with pale yellow color and mild smell.

Calculating the values of pH, titratable acidity, specific gravity, and microchemistry screening test are the primary components of chemical measurements. Urine pH can reflect the risk of urine stone formation, and the normal pH range is from 4.5 to 8.0, while the value between 5.7 and 6.3 has a lower risk of stone formation.[6] Titratable acid is excreted into urine as a result of acid-base balance. Titratable acidity is a good indicator of kidney function and acid retention, which has clinical significance for the treatment to patients with chronic kidney disease.[7] Normally, the titratable acidity ranges from 20 to 50 mEq/L, which would be largely influenced by diet.[8] Urine specific gravity uses a urinometer to estimate the ratio of urine solution to its equal volume of water, which is clinically applied to evaluate the kidney capacity of concentrating or diluting the glomerular filtrate.[9] Its normal range is 1.005 to 1.030 for a 24-hour urine collection.[10] Microchemistry screening test uses urine reagent strips to evaluate the presence of blood, ketones, glucose, protein, and its pH value. It is an effective and efficient measurement to diagnose a series of diseases such as diabetes mellitus, hematuria, proteinuria, etc. The test for healthy individuals shows negative results, while it could be affected by dietary

intake. False-positive or false-negative may also occur due to contamination, presence of ascorbic acid and many other reasons.[9]

Urinary calcium level is usually measured to evaluate the kidney function and calcium balance in our body, where the abnormal value may indicate a higher risk of kidney stones and abnormal function of parathyroid gland.[11] Normal ranges vary between 50 and 300mg/day depending on the dietary intake.[12] Urine creatinine is also an important indicator to evaluate the kidney function. It is excreted entirely through urine, and a decreased creatinine level may result from impaired kidney function. Normal range of creatinine for 24-hour urine is from 500 to 2000 mg, which could be influenced by subject's age and lean body mass.[13]

The purpose of this experiment was to evaluate the urine components by using physical and chemical measurements of the 24-hour and random-single urine samples from the a 21-year-old non-athlete male who is in healthy condition. Urine volume, color, and odor was recorded through observation; pH and titratable acidity were measured by pH meter; specific gravity was measured by urinometer to assess kidney function and dehydration status; microchemistry screening test was applied to measure the presence of blood, ketones, glucose, protein and pH value, so as to screen kidney or systemic diseases. Urinary calcium and creatinine levels were measured by spectrophotometry, and the subject's lean body mass was further estimated based on the creatinine value of the 24-hour urine with its percent recovery. It is anticipated that all the measured values from the subject are within the normal range.

Methods

The subject was a 21-year-old non-athlete male, self-reported in healthy condition. 24-hour urine sample was collected in collection bottles on September 23, 2020. During the 24-hour

urine collection, he consumed 15 cups water, 1½ cups iced tea, 1½ cups cranberry juice, 1 cup apple, 3 oz eggs, 1 cup rice, 4 tbsp avocado, 4 tbsp cucumber, 4 tbsp green peas, 5 oz beef, 1 oz pork, 2 oz chocolate, 8 oz banana, 2 cups yoghurt, and 2 cups watermelon. Random-single urine sample was collected on September 22, 2020, which was the first morning urine. The subject ate similar categories of food during the days when urine samples were collected.

Urine volumes for 24-hour and random samples were measured according to the mark of the collection bottle, where 200 mL of each were transferred into beakers. Urine color and odor were then observed from beakers.

pH Value was measured by pH meter, and the titratable acidity was calculated from the volume of standardized 0.1N NaOH added to titrated urine sample for reaching pH 7.4.

$$\mathbf{mEq\ (NaOH)} = \mathbf{V\ (NaOH, mL)} \times \mathbf{0.1\ (mEq/mL)} \times \frac{\mathbf{V\ (entire\ sample)}}{\mathbf{V\ (titrated\ sample)}}$$

Urinometer was used to calculate urine specific gravity. Calibration for the urinometer is necessary before reading, and the specific procedure was illustrated in the Laboratory Manual (3a).[14]

Microchemistry screening test was conducted by using reagent strips that reflect the presence of blood, ketones, glucose, protein, and the value of pH. Methods about how to use the reagent strip was also described in the Laboratory Manual (3a).[14]

Urinary calcium level was calculated from the 24-hour urine sample by using the *Cayman* kit. Sample was diluted 1:1 with calcium assay buffer and was added to calcium indicator o-cresolphthalein in the 96-well plate. Calcium standard curve was determined, and the absorbance for 0 mg/dL calcium standard was corrected to zero. The concentration of urinary calcium was thus calculated with its dilution factor, and the total amount of calcium excretion was obtained by multiplying the total volume of 24-hour urine.

m (total Ca^{2+}) = c[Ca^{2+}] (mg/dL, in the well) × 2 (dilution factor) × V (mL, total urine) ÷ 100

Urinary creatinine level for urine samples (24-hour sample, random sample, 24-hour + standard sample) were calculated based on the creatinine standard curve, which was corrected and adjusted by initial and final absorbances. The percent recovery for 24-hour urine sample was calculated to obtain the total amount of creatinine. Specific steps of creatinine calculations could be found in Laboratory Manual (3b).[15]

$$\%\mathbf{Recovery} = \frac{[\mathbf{24hour + standard\ sample}](\mathbf{mg/dL}) \ - \ [\mathbf{24hour\ sample}](\mathbf{mg/dL})}{\mathbf{6}\ (\mathbf{mg/dL, standard})}$$

m (total creatinine for 24-hour urine sample)

= c[creatinine] (mg/dL, in the well) × 20 (dilution factor) × V (mL, total urine) ÷ 100 ÷ %recovery

m (total creatinine for random urine sample)

= c[creatinine] (mg/dL, in the well) × 20 (dilution factor) × V (mL, total urine) ÷ 100

Lean body mass (LBM) was then calculated based on the equation in Laboratory Manual (3b).[15]

$$\mathbf{LBM\ (kg)} \ = \ (\mathbf{0.02908 \times mg\ creatinine\ excreted\ per\ day}) + \mathbf{7.38}$$

Results

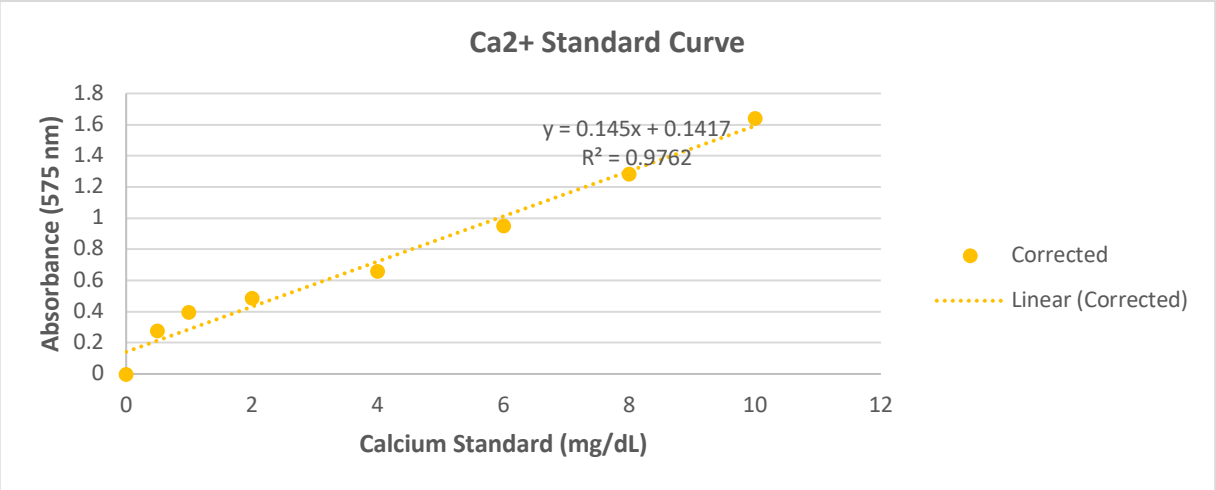
The subject's urine volume, color, odor, pH, titratable acidity, specific gravity, microchemistry screening test results are shown in **Table 1** below. Titratable acidity of both 24-hour and random urine samples are lower than expected, trace amount of protein was detected, and other values are within the normal range.

Table 1. Physical and chemical examination results for 24-hr and random-single urine samples

	24-hr urine sample	Random-single urine sample	Normal Range
Urine volume (mL)	1800	210	800 - 2000 (24-hr)
Urine color	Transparent, pale yellow	Transparent, pale yellow	pale yellow to deep amber
Urine odor	Faint, aromatic odor	Faint, aromatic odor	Faint, aromatic odor
pH (pH meter)	6.57	6.56	4.5 - 8.0
Titratable acidity (mEq/L)	3	14	20 - 50
Specific gravity	1.006	1.017	1.005 - 1.030
Blood	Negative	Negative	Negative
Ketones	Negative	Negative	Negative
Glucose	Negative	Negative	Negative
Protein	Negative	Trace	Negative
pH (reagent strip)	6.5-7.0	6.5-7.0	4.5 - 8.0

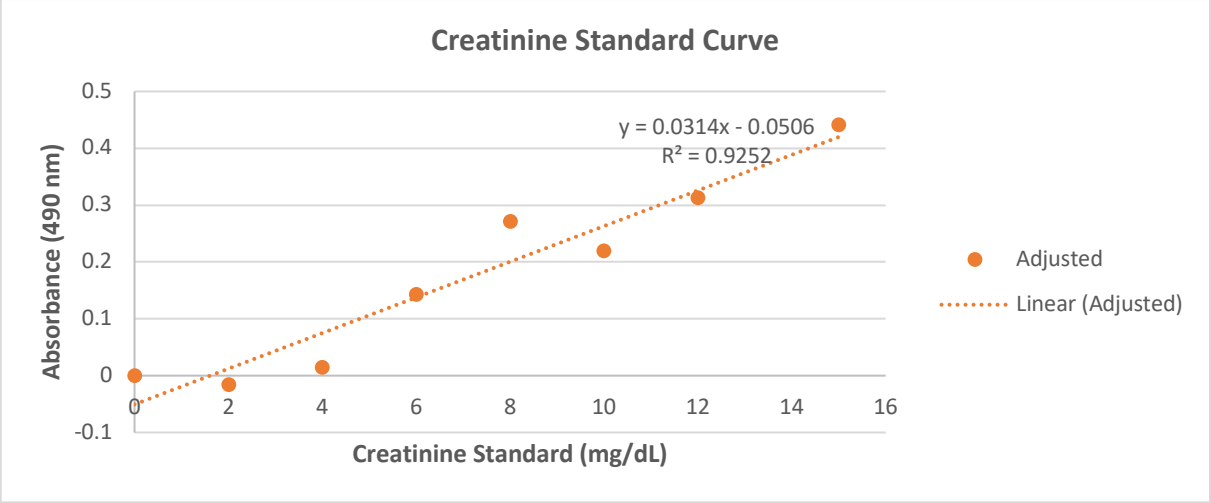
The calcium standard curve is shown in **Chart 1**. The standard curve equation is $y = 0.145x + 0.1417$, and R^2 value is 0.9762. The total calcium excreted per day is **202.68 mg**, which is within the normal range (50 - 300mg).

Chart 1. Calcium Standard Curve. The concentration of calcium in well is 5.63 mg/dL.



The creatinine standard curve is shown in **Chart 2**. The standard curve equation is $y = 0.0314x - 0.0506$, and R^2 value is 0.9252. The percent recovery for 24-hour sample is **239.38%**. Total creatinine excreted in 24-hour urine sample is **101.052mg**, and the total creatinine in random sample is **370mg**. The lean body mass is **10.32kg** calculated from the LBM equation with the amount of total creatinine in 24-hour urine sample.

Chart 2. Creatinine Standard Curve. The concentrations of creatinine in wells for 24-hour sample, random sample, and 24-hour+standard sample are 0.672mg/dL, 8.809mg/dL, 15.035mg/dL.



Discussion

The results have shown that the subject's urine volume, color, odor, pH, and specific gravity are in the normal range, which is consistent with the expected results. Titratable acidity for 24-hour and random-single urine samples are 3mEq/L and 14mEq/L, which are lower than the normal range. There is a trace amount of protein in the random-single urine sample. The total calcium excretion per day is 202.68mg, which is in the normal range. The total creatinine in 24-hour urine is 101.052mg, which is far below the normal range (500 - 2000 mg/day). These urine components were measured by different physical and chemical methods, and the quality of some results needs to be discussed.

The specific gravity was measured by urinometer, which effectively reflects the solutes amount in urine. In this experiment, urinometer was used to measure both the 24-hour and random urine samples. There is a relatively large variation between two values (1.006 and 1.017), which may indicate that the random urine sample is more concentrated. As the temperature change would influence the value of specific gravity, calibration is necessary for urinometer to obtain the accurate results.

Titratable acidity is expected to be higher than the measured results and there is a large variation between the results of 24-hr urine sample and random urine sample. Errors may occur when the pH meter did not respond sensitively to the addition of NaOH.

Microchemistry screening test is a quite effective measurement to screen certain diseases, based on several chemical reactions on the reagent strip with human blood, ketones, glucose and protein. In this experiment, trace amount of protein was found in the random urine sample. High protein meals may lead to this result.

Calcium and creatinine levels were measured by the *Cayman* detection kit. In this experiment, the total calcium level is consistent with normative values, while the total creatinine in 24-hour urine is much lower than expected. The value of lean body mass (10.32kg) also made the whole results doubtful. Theoretically, total creatinine should be higher in 24-hour urine sample than it is in random urine sample, as the random urine sample has a lower volume and didn't consider its percent recovery. In addition, the percent recovery of 24-hour urine sample is abnormally high (239.38%), so the result for creatinine is less accurate and needs to be remeasured. Errors may occur during the pipetting process towards the 96 well plate, as the volume measured was pretty small.

Conclusion

The purpose of this experiment was to analyze the subject's urine components through a series of physical and chemical measurements. The study has found that the subject's urine volume, color, odor, pH, specific gravity, microchemistry screening test, and urinary calcium are within the normal range, while the titratable acidity and creatinine levels are much lower than expected. In general, most of these measurements could reflect the health status of the subject and could be applied as initial screening or diagnosis for certain diseases. However, the experiment didn't demonstrate its capacity to ensure accuracy and precision, especially for the measurement of urine creatinine and its titratable acidity. Therefore, repeated measurements and further analysis are needed to obtain more accurate values of urine components.

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