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# Laboratory Trial of Protein Determination in Urine Using Different pH Values of Acetic Acid and Acetate Buffer Method

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**Abstract**

The determination of protein in urine is important in clinical examination along with other parameters in urine. The presence of protein in urine can be interpreted that there is a disorder in kidney. Acid and heat coagulations method is still widely used in many areas to determine protein in urine. In this method, the characteristic of protein that will precipitate in the presence of acid or if exposed to heat is deployed to gain information about the amount of protein. The geater amount of protein, the more prominence is the coagulation. Urine pH also varies according to the condition, classic acidosis will give an acidic urine and the presence of ammonium producing bacteria can cause basic urine. In this research acetic acid method with 6% of $CH_3COOH$ and pH value of 2.9 and buffer acetic with pH 4.5 are used to determine the certain amount of protein (+3 value, corresponds with 2-4 mg/dL protein in urine) in varied pH values of urine samples. In order to compare the results, first in control urine with pH 6.8 the results of both methods is compared with Mann-Whitney test and shows no significant different, then the Kruskall-Wallis test is used to compare the results in other pH values to control and the test is shown also there are no significant difference. This shows that either acetic acid at pH 2.9 or acetic buffer at pH 4.5 can be used to determine protein amount in urine.

**Keywords**

Proteinuria, acetic acid, acetate buffer, urine pH, buffer

## INTRODUCTION

Under normal physiological state, urine is expected to be protein free. High molecular weight proteins in plasma (e.g., albumin and globulin) could not pass through the filtration membrane due to the effects of the size barrier and charge barrier of the glomerular capillary filtration membrane. Low molecular weight proteins (e.g., β2-microglobulin (β2-M), α1-microglobulin (α1-M), and lysozyme), however, can freely pass through the filtration membrane, although the filtration amount is low and 95% of these proteins are reabsorbed when entering the proximal convoluted tubule (1,2). The final urine protein content is

therefore low (only 30-130 mg/24h) and consists primarily of plasma albumin (40%), immunoglobulin fragments (15%), other plasma proteins (5%), and urinary system-originating tissue proteins (40%). The protein concentration in a random urine sample is 0-80 mg/L, and the results of qualitative tests for urokinase protein are typically negative. When the urine protein exceeds 150 mg/24h or the concentration is above 100 mg/L, the result for the qualitative protein test becomes positive. This is known as proteinuria (3).

Proteinuria is common finding in chronic renal failure patients and current evidence indicates that the presence of proteinuria is an early marker of an increased risk of progessive kidney disease, poor cardiovascular and death (2).

As the measurement and sampling procedures for proteinuria assessment have not been standardized yet, it is of clinical importance to take into account different types of urinary proteins, albumins, laboratory techniques, and urine sampling methods in order to have the best approach for an individual patient (4).

Total urinary protein can be assessed using dipstick, precipitation, and electrophoresis methods. Urine specimen for proteinuria assessment can be obtained either from a timed collection or a spot urine sample. Nevertheless, currently spot urine protein- or albumin-to-creatinine ratios are preferred to a 24-hour urine sample in routine practice (4). The proteinuria is commonly assessed by the heat and acetic acid test. Now dipstick test is replacing the old methods (5).

Heat coagulation test may be used in under-resourced settings as an alternative to dipstick testing or other methods that are unavailable or too costly. A test tube is filled to two-thirds with urine. A few drops of dilute acetic acid are added to make the urine sample acidic. The upper part of the test tube containing urine is heated (but not boiled) over a burner. Acidic environment is to ensure that the coagulation formed is protein because on heating, phosphates also coagulate but dissolves in acidic environment (6).

The result is considered to be negative (no protein presents in sample) if there is no cloud in solution after the test, a +1 (0.1 g/dL) result if there is a definite cloudiness, if held against a typed papers, letters typed can be seen through the cloud in upper part of the test tube, +2 (<0.3 g/dL) if there is definite cloudiness, if held against the typed paper, letters typed are seen as faint lines in the backgound at top of the test tube, +3 (0.3-1.0 g/dL) if there is a definite cloudiness, if held against the typed paper, letters typed are seen as faint lines in the backgound at top of the test tube, +4 (>1.0 g/dL) if there is a thick solid precipitation, clot present at top of the test tube (6,7).

The newer method after heat and acid coagulation is dipstick method to detect

proteinuria. The reaction is based on the phenomenon known as the 'protein error' of pH indicators (a dye) where an indicator that is highly buffered will change color in the presence of proteins (anions) as the indicator releases hydrogen ions to the protein. The dye is acid-base indicators such as tetra bromophenol blue. When the dye is buffered at pH 3, it is yellow; the addition of increasing concentration of protein changes the color to geen then to blue. The developed color is compared with a color chart which allows protein concentration to be gaded from trace to +4, corresponding to concentrations from 1 to 10 mg/dL to geater than 500 mg/dL (8).

At a constant pH, the development of any geen color is due to the presence of protein. Colors range from yellow to yellow-geen for negative results and geen to geen-blue for positive results. But the clinical judgement is required to evaluate the significance of trace results. A color to be defined as proteinuria differ from each manufacturer, but usually geater than 30 mg/dL indicates significant proteinuria (8).

Urine protein mainly consists of albumin. This protein can reversibly and drastically change its conformation when exposed to changes in solution pH (transitions occurring at pH 2.7, 4.3, 8, and 10) (9).

As for urine, the normal pH range is 5 to 7, with low urine pH can be caused by high protein diet because the increased endogenous acid production from sulfur-containing amino acids or metabolic acidosis (e.g., chronic diarrhea) while high urine pH (usually >7) is caused by metabolic alkalosis (e.g., vomiting), distal renal tubular acidosis, urea-splitting organisms (e.g., Proteus), urine that is infected will become alkaline over time due to formation of ammonia ($NH_3$) from bacterial urease, urine that is exposed to air for a long time can also have elevated pH due to loss of $CO_2$ from urine (8).

So the aims of this study was to examine the results from acetic acid test with using 6% of acetic acid (pH 2.9) and acetate buffer (pH 4.5) of urine in varied pH values or urine samples with addition of known amount of protein to mimic proteinuria conditions.

## MATERIALS AND METHODS

Apparatus used in this research are 100 mL beaker glasses, Dirui DR-7000E photometer, stirrer, micropipette, spiritus burner, test tubes and test tube-rack, Manti Lab MT-103 pH meter, FanMed 80-i centrifuge, and its centrifuge tubes.

Materials used are Biuret Reagent. Biuret reagent is prepared by dissolved 1.5 g of copper sulfate pentahydrate ($CuSO_4.5H_2O$) and 6 g of sodium potassium tartrate ($KNaC_4H_4O_6.4H_2O$) in 500 mL of water, and add 300 mL of 10% (w/v) of NaOH, and add 1 g of potassium iodide and make a 1L solution (10).

Acetic acid ($CH_3COOH$) 6%, sodium acetate ($CH_3COONa$), acetate buffer (pH 4.5) is made with 1.544 g of sodium acetate anhydrous and 0.076 g of acetic acid to make 200mL of solution. pH is checked with Manti Lab MT-103 pH-meter and adjusted with HCl or NaOH to achieve pH of 4.5.

Serum and urine were taken from four healthy volunteers from students of Sekolah Tinggi Analis Bakti Asih wtih normal plasma protein level and negative test of protein in urine.

To make serum, 3 mL of blood is drawn from vein and put in centrifuge tube for 10 minutes before being centrifuged at 1500 rpm for 15 minutes to separate serum from blod clot. Carefully serum is pipetted and tested for protein with Biuret method. Twenty microliters of serum is added to 1mL of Biuret reagent. The mixture is incubated for 5 minutes at room temperature (25$^oC$) then put in photometer. The reading of absorbance at 546 nm is compared to absorbance of protein standard solution and protein concentration in serum is calculated. As much as 5 g/dL of protein in serum is obtained.

Urine sample that is used is a random time and collected in a clean and dry plastic container with a lid. The urine specimen is tested with two methods to check its protein contents then proceed to the addition of serum to make simulated urine that contains protein at +3 (4 mg/dL). For each of pH group, a 40 mL portion of urine is needed.

Simulated urine with protein that can be detected by heat and acid coagulation is achieved by adding serum with the known concentration of protein to urine, the result is urine sample with protein concentration is 4 mg/dL (+3).

Urine batch is divided to make urine samples with different pH by adding ammonium hydroxide or citric acid (pH range is 6, 6.5, 6.8, 7, and 7.5). We made four replications for each method in each pH value.

Simulated urine with pH 6.8 is chosen as normal pH. To compare the median value between acetic acid results goup and buffer acetate results goup at the normal condition (control at pH 6.8), the Mann-Whitney test is used, then Kruskal-Wallis test is used for statistical treatment of these data (among three groups, i.e.; acetic acid results, acetate buffer result at each pH , and control) because it analyzes whether there is a difference in the median values of three or more independent samples (11).

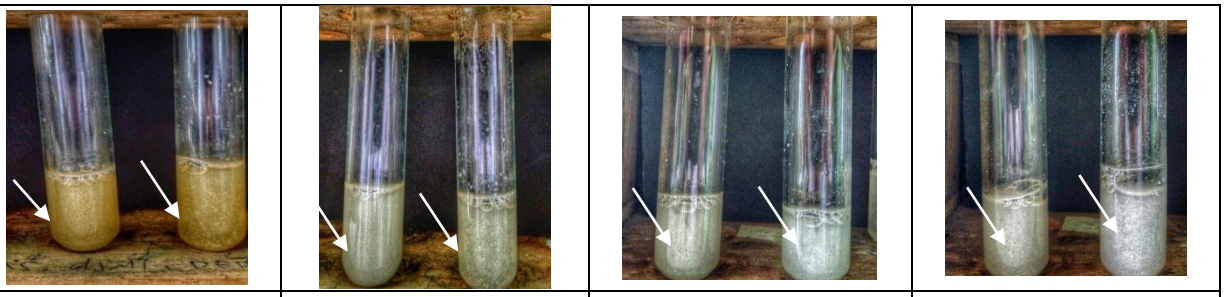
All experiment is conducted in Chemistry Laboratory of Sekolah Tinggi Analis Bakti Asih Bandung, West Java-Indonesia during March until May 2019 and this study was approved by the Ethics Committee of Sekolah Tinggi Analis Bakti Asih Bandung.

## RESULTS

The results of this study can be seen in the following Table 1:

**Table 1.** Results of Experiments

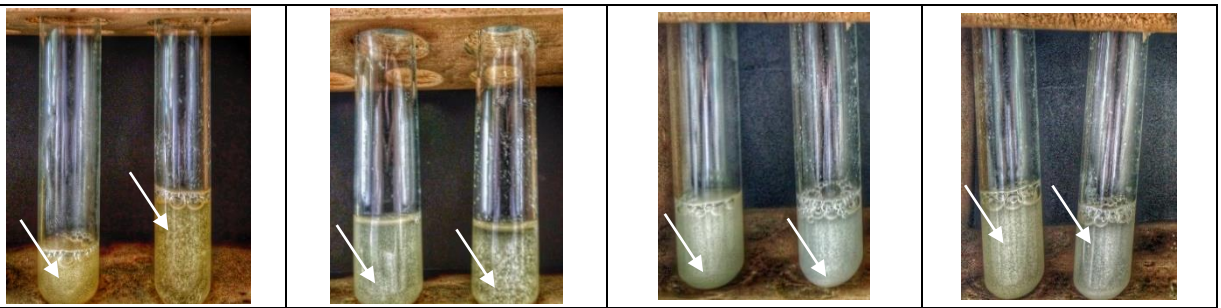
| Treatment | Test | Results | | | |
|---|---|---|---|---|---|
| | | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
| **Blank** | Acetic Acid | ( - ) | ( - ) | ( - ) | ( - ) |
| | Acetic Buffer | ( - ) | ( - ) | ( - ) | ( - ) |
| **pH 6.0** | Acetic Acid | ( +++ ) | ( +++ ) | ( +++ ) | ( +++ ) |
| | Acetic Buffer | ( +++ ) | ( +++) | ( +++ ) | ( +++ ) |
| **pH 6.5** | Acetic Acid | ( +++ ) | ( ++) | ( +++ ) | ( +++ ) |
| | Acetic Buffer | ( +++ ) | ( +++ ) | ( +++ ) | ( +++ ) |
| **pH 6.8** | Acetic Acid | ( ++ ) | ( ++ ) | ( ++ ) | ( +++ ) |
| | Acetic Buffer | ( ++ ) | ( +++ ) | ( +++ ) | ( +++ ) |
| **pH 7.0** | Acetic Acid | ( ++ ) | ( ++ ) | ( ++ ) | ( +++ ) |
| | Acetic Buffer | ( ++ ) | ( ++ ) | ( ++ ) | ( +++ ) |
| **pH 7.5** | Acetic Acid | ( ++ ) | ( ++ ) | ( ++ ) | ( +++ ) |
| | Acetic Buffer | ( ++ ) | ( ++ ) | ( ++ ) | ( ++ ) |



| a = (+++)<br>b = (+++) | a = (+++)<br>b = (+++) | a = (+++)<br>b = (+++) | a = (+++)<br>b = (+++) |
|---|---|---|---|

a; left test tube is simulated urine sample with acetic acid.
b; right test test tube is simulated urine sample with acetic buffer

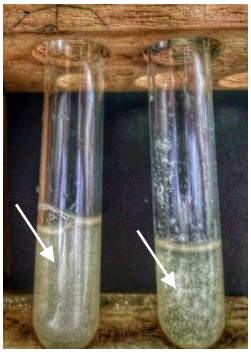
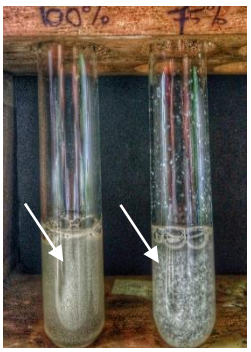
**Fig 1.** Results for Simulated Urine Samples at pH 6.0. White Arrows Indicated The Cloud/Precipitation of Protein.



| a = (+++)<br>b = (+++) | a = (++)<br>b = (+++) | a = (+++)<br>b = (+++) | a = (+++)<br>b = (+++) |
|---|---|---|---|

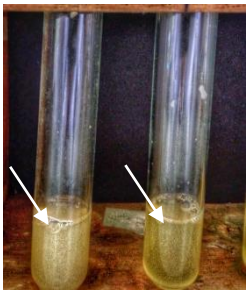
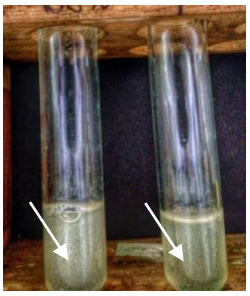
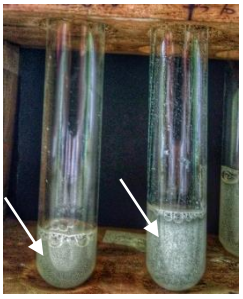
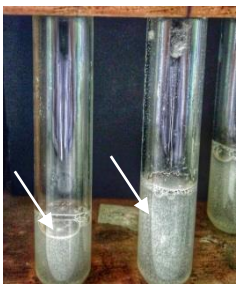
a; left test tube is simulated urine sample with acetic acid.
b; right test test tube is simulated urine sample with acetic buffer

**Fig 2.** Results for Simulated Urine Samples at pH 6.5. White Arrows Indicated The Cloud/Precipitation of Protein.

| | | | |
|---|---|---|---|
|  |  |  |  |
| a = (+++)<br>b = (++) | a = (+++)<br>b = (++) | a = (++)<br>b = (++) | a = (++)<br>b = (+++) |

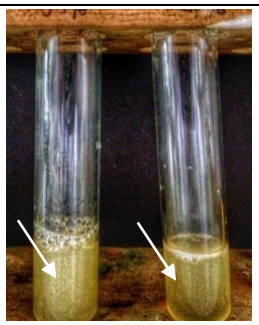
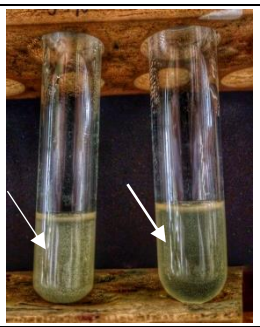
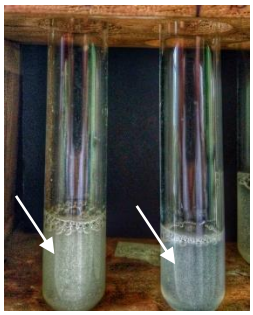
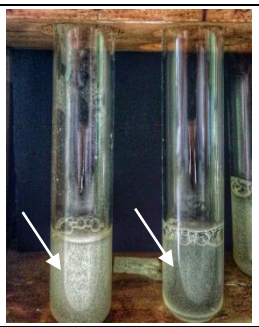
a; left test tube is simulated urine sample with acetic acid.
b; right test tube is simulated urine sample with acetic buffer

**Fig 3.** Results for Simulated Urine Samples at pH 6.8. White Arrows Indicated The Cloud/Precipitation of Protein.

| | | | |
|---|---|---|---|
|  |  |  |  |
| a = (++)<br>b = (++) | a = (++)<br>b = (++) | a = (++)<br>b = (++) | a = (+++)<br>b = (+++) |

a; left test tube is simulated urine sample with acetic acid.
b; right test tube is simulated urine sample with acetic buffer

**Fig 4.** Results for Simulated Urine Samples at pH 7.0. White arrows indicated the cloud/precipitation of protein.

| | | | |
|---|---|---|---|
|  |  |  |  |
| a = (+++)<br>b = (++) | a = (++)<br>b = (++) | a = (++)<br>b = (++) | a = (+++)<br>b = (++) |

a; left test tube is simulated urine sample with acetic acid.
b; right test test tube is simulated urine sample with acetic buffer

**Fig 5.** Results for Simulated Urine Samples at pH 7.5. White Arrows Indicated The Cloud/Precipitation of Protein

The Mann-Whitney test between acetic acid result and acetate buffer result at control pH (6.8) shows no significant difference,that means we can say both group gave the same result at normal pH. Then we continued to the Kruskall-Wallis test. It was used to compare the median values among groups in each pH compared to control. The test also shows there is no significant differences.

## DISCUSSION

The determination of urinary protein is important for its significance use in clinical diagnosis (12). The rapid test can be performed by dipstick but the heat and acid coagulation test are still widely used.

In determining proteinuria itself, classically this is done by a 24-hour collection, but as creatinine is excreted at a constant rate, a ratio of urine albumin to creatinine or protein to creatinine is sufficient in most patients (13). Nevertheless in urinalysis if the protein persists and the amount is significant one can suspect the patients has proteinuria symptoms.

In this experiments it has been shown that heat and acid coagulation test can be used in wide range of pH (Figure 1-5). Since there can be variation in urine pH from time to time because of many reasons among other the bacteria that produces ammonium hydroxide that can cause basic urine, or acidosis case which can make urine acidic. This implies to the environment of protein in urine. Since each protein has a definite isoelectric point and albumin which is the dominant protein in urine has isoelectric point of 4.5 then the change of pH nearing to that point will cause albumin to coagulate which we can correspond to the amount of protein presents in urine (14).

With +3 protein (0.2-0.4 g/dL) in urine samples, it has been shown in this experiment that acetic acid test is in accordance with acetic buffer test which used the prominent properties of protein that will coagulate in the presence of heat and/or acid. This process if known as denaturation of protein. That means the change of protein environment will cause several damages in protein structures. The clear sign of denaturation is precipitation in which soluble protein has lost several bonds in its structure and protein precipitates.

As for these two methods, it has been shown that they can be used in many pH values of the urine samples. A method to be a useful method must endure and still gives a reliable result in several clinical conditions.

## CONCLUSIONS

According to the results of experiments and Kruskall-Wallis test, the determination of protein in urine at pH 6.5 to 7.5 can be done with acetic acid test with 6% (pH 2.9) or acetate buffer (pH 4.5). Both of method were give +3 positive value which correspondent of 0.2-0.4 g/dL of protein.

## CONFLICT OF INTEREST

There are no conflicts of interest.

## REFERENCES


1. Stankov S V. Definition of Inflammation, 1. Zhang A, Huang S. Progress in pathogenesis of proteinuria. Int J Nephrol. 2012;2012.
2. Prakash M, Phani NM, Kavya R, Supriya M. Urinary peptide levels in patients with chronic renal failure. Online J Heal Allied Sci. 2010;9(3):1–3.
3. Hong DSC, Oh IH, Park JS, Lee CH, Kang CM, Kim GH. Evaluation of Urinary Indices for Albuminuria and Proteinuria in Patients with Chronic Kidney Disease. Kidney Blood Press Res. 2016;41(3):258–66.
4. Toblli JE, Bevione P, Di Gennaro F, Madalena L, Cao G, Angerosa M. Understanding the mechanisms of proteinuria: Therapeutic implications. Int J Nephrol. 2012;2012.
5. Vaidyanathan K. Textbook of Biochemistry for Medical Students. Textb Biochem Med Students. 2016;(August).
6. Beaufils MA an. MMM von D. The FIGO Textbook of Pregnancy Hypertension. Vol. 6, Nephrologie et Therapeutique. 2016. 200–214 p.
7. Mauliddina J. Detecting Proteinuria: A Comparison of Diagnostic Tests. Paediatr Indones. 2011;51(4):207–12.
8. Kwong T, Robinson C, Spencer D, Wiseman OJ, Karet Frankl FE. Accuracy of urine pH testing in a regional metabolic renal clinic: Is the dipstick accurate enough? Urol Res. 2013;41(2):129–32.
9. Pergande MR, Cologna SM. Isoelectric point separations of peptides and proteins. Proteomes. 2017;5(1).
10. Janairo, Gerardo, Linley Sy, Marianne, Yap, Leonisa, Llanos-Lazaro, Nancy, Robles J. Determination of the Sensitivity Range of Biuret Test for Undergraduate Bio...: Discovery Service for Universiti Tunku Abdul Rahman. e-Journal Sci Technol. 2011;6(5):p77-83.
11. Nahm FS. Nonparametric statistical tests for the continuous data: The basic concept and the practical use. Korean J Anesthesiol. 2016;69(1):8–14.
12. Matica J. Disease : National Recommendations. 2017;27(1):153–76.
13. Chen YT, Hsu HJ, Hsu CK, Lee CC, Hsu KH, Sun CY, et al. Correlation between spot and 24h proteinuria: Derivation and validation of equation to estimate daily proteinuria. PLoS One. 2019;14(4):1–12.
14. Audain E, Ramos Y, Hermjakob H, Flower DR, Perez-Riverol Y. Accurate estimation of isoelectric point of protein and peptide based on amino acid sequences. Bioinformatics. 2016;32(6):821–7.