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Performance Test of Homemade Control Materials from Addition and Spike Placebo Simulation on Urine Chloride Examination with Fantus Method

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ABSTRACT

The background of this research was to examine the performance of homemade urine control materials on urine chloride examination. This study was aimed to test the performance of homemade control materials from addition and spike placebo simulation on urine chloride examination using the Fantus method. This research applied an experimental research method. The stages of the study started with standardization of $AgNO_3$ solution, preparation of urine control materials from addition and spike placebo simulation, as well as calculation of accuracy (%R), bias (%d) and coefficient of variation (%CV). The results showed that the normal and pathological levels of accuracy in the urine control materials from addition were 109.55% and 104.30%, while the accuracy values (%R) for urine control materials from spike placebo simulation, for the normal and pathological levels, were high, 110.07% and 104.54%. The bias values (%d) obtained in the urine control materials from addition with normal and high pathological levels were 9.55% and 4.30% bias, while the bias values (%d) in the urine control materials from spike placebo simulation with normal and high pathological levels were 10.07% and 4.54%. The coefficient variation (%CV) values in urine control materials from addition at normal and pathological high levels were 3.75% and 3.07%, while the %CV values in urine control materials from spike placebo simulation at normal and high pathological levels were 4.55% and 3.11%. This study concludes that based on the accuracy (%R) and bias (%d) parameters, the homemade control materials from addition and spike placebo simulation have relatively good performance for urine chloride examination using the Fantus method. Meanwhile, based on the parameter of coefficient of variation (%CVV), the homemade control materials from the addition and spike placebo simulation have slightly lower performance for examining urine chloride with the Fantus method.

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INTRODUCTION

The chloride level in the body is determined by the amount of chloride that enters and leaves the body. The chloride entering the body is influenced by the amount and type of food intake. Normal adults consume an average of 50-200 mmol chloride per day and excrete chloride with feces about 1-2 mmol per day. Excessive sweating can result in chloride losses of up to 200 mmol per day (Yaswir R & Ferawati I, 2012).

Chloride has a molecular weight of 35.5 g/mol and serves as the main anion for the body (Yunos et al., 2010). Excessive excretion of chloride in the urine is also found in patients with Bartter syndrome and hyperactivity of the renin-angiotensin-aldosterone system (Graziani et al, 2010). A depletion or a decrease in chloride level prevents bicarbonate secretion in the cortical collecting ducts, while an increase in bicarbonate reabsorption in the outer medullary collecting ducts maintains chloride-responsive alkalosis. Administration of chloride increases chloride delivery to the collecting ducts and rises urinary bicarbonate excretion by increasing secretion and reducing reabsorption. Chloride-resistant metabolic alkalosis is mostly a compromised syndrome characterized by absolute or marked mineralocorticoid excess. However, the use of active diuretics, which inhibit compensatory renal chloride retention, is a notable exception. (Heffner et al, 2009).

The amount of chloride in the extracellular fluid is 88% and in the intracellular fluid is 12%. The diverse chloride levels in interstitial fluid and intracellular fluid are attributed to the dissimilarity in tension on the outer and inner surfaces of the cell membrane (Klutts & Scott, 2006). Urinary chloride examination is important to monitor the amount of chloride daily excretion. The amount of released chloride per day that exceeds the amount of chloride entering the body will cause hypochloremia. This situation is triggered by the addition of excess water to the extracellular fluid as well as chloride deficiency. Hypochloremia may also occur in disorders associated with bicarbonate resistance, such as chronic respiratory acidosis. Meanwhile, if the amount of chloride intake exceeds the chloride release, hyperchloremia may occur. This disorder can be found in the cases of renal tubular acidosis, acute renal failure, dehydration, and metabolic acidosis (Yaswir & Ferawati, 2012).

Laboratories play a vital role in health services, especially in establishing a diagnosis of disease, determining the cause of disease, supporting an early warning system, monitoring treatment, maintaining health, and preventing disease (Permenkes, 2013). Examination of chloride in urine in the laboratory includes the application of the Fantus (argentometry) method (Musser, 1961). In this examination, silver nitrate is added with potassium chromate indicator, and this combination will react with urine chloride to form a brick-red precipitate. Meanwhile, urine chloride examination is performed by examining the control material to maintain quality assurance.

Commercial urine control materials, especially those used for chloride testing, are currently relatively difficult to obtain. Therefore, an alternative to commercial urine control materials is needed by making urine control materials. Several methods that can be applied to produce the materials are urine control preparation (spike placebo simulation) and addition control material preparation (Siregar et al., 2018).

The spike placebo simulation method can be used to produce analytes with definite levels to analyze the accuracy (%R) of the examination results easily (Riyanto, 2014). The addition method is performed by adding a certain amount of analytes to the analyzed samples. Accuracy (%R) is determined by estimating the percentage of the added analytes found. Research on the production of urine control materials, especially those used for the examination of urine chloride using the Fantus method, has not been widely done. Thus, it is necessary to research the production of control materials from addition and spike placebo simulation for the examination of urine chloride

using the Fantus method to determine the performance of the control materials.

MATERIALS AND METHODS

Materials and Equipment

The tools used in this study were a glass funnel, spatula, stirring rod, test tube (Iwaki), test tube rack, dropper pipette, 1mL and 10mL measuring pipettes (Pyrex), Ohaus PA214 balance, 100mL beaker (Pyrex), 100mL burette (Pyrex), and 250 mL Erlenmeyer (Pyrex).

The materials needed in this study were urine samples, standard solution of 0.1N NaCl p.a. (Merck), AgNO3 2.9% (0.17N) p.a. (Merck), K2CrO4 20% p.a. (Merck), and distilled water (aquadest).

Research Procedure

Standardization of $AgNO_3$ Solution with 0.1N NaCl

The procedure for standardizing the AgNO3 solution began with adding 10 mL of 0.1 N NaCl solution into an Erlenmeyer flask using a pipette. Then, 1 mL of 20% K_2CrO_4 was added into the solution and the $AgNO_3$ solution was put into the burette. The 0.1 N NaCl solution was titrated with $AgNO_3$ until a brick-red precipitate was formed. The titration was repeated three times. The normality of $AgNO_3$ was calculated from the results of the titration (SNI 06-6989.19-2004).

Preparation of Urine Chloride Control Materials with Spike Placebo Simulation Method

The preparation of normal (180 mEq/L) and pathologically high (300 mEq/L) urine chloride control was started by adding 1N NaCl standard solution into two 100mL volumetric flasks of 18mL and 30mL. Then, distilled water was added until reaching the mark and homogenized.

Preparation of Urine Chloride Control Materials by Addition Method

The preparation of urine chloride control by addition method for normal and pathological high levels began with filling two test tubes with 10 drops of urine using a dropper. Then, 10 drops of standard chloride solution of 180 mEq/L were added into the first tube and 300 mEq/L into the second tube. About 2 drops of 20% K_2CrO_4 solution were put into each tube with a 1mL measuring pipette, then homogenized. After that, the AgNO3 solution was dripped until a permanent brick-red color was formed. The control urine chloride level was estimated. The number of drops of silver nitrate solution used equaled grams of chloride per liter of urine.

Determination of Urine Chloride Levels using Addition and Spike Placebo Simulation

The determination of urine chloride levels began with filling a test tube with 10 drops of urine control material using a dropper and adding 2 drops of 20% K_2CrO_4 solution with a 1mL measuring pipette. The solution was then mixed. After that, 0.17 N (2.9%) AgNO3 solution was dripped until a brick red color was formed and the chloride content was measured. The number of drops of silver nitrate solution used was equivalent to grams of chloride per liter of urine. The results of this examination were used to calculate the accuracy (%R), bias, and coefficient of variation.

RESULTS AND DISCUSSION

The Purpose of Urine Chloride Examination

The urine chloride test is performed to evaluate the urine chloride concentration to differentiate between chloride responsiveness and chloride-resistant metabolic alkalosis. If the kidneys detect a reduced effective circulating volume, they will immediately reabsorb Na+, HCO_3- and chloride via the activated renin-angiotensin aldosterone system, thereby reducing urinary chloride concentrations. Although the loss of gastric acid and diuretics can result in volume depletion and secondary alkalosis, chloride content (either in the form of NaCl or KCl) will improve metabolic alkalosis, even if volume depletion continues (Rossen et al. 1998).

Standardization of $AgNO_3$ Solution with 0.1N NaCl

Standardization is carried out to define the actual content of the secondary standard solution that has been made so that the examination produces the proper data (Padmaningrum, 2006). In this study, $AgNO_3$ is a type of secondary standard solution that must be standardized with a primary standard of NaCl when used to measure urine chloride levels. The $AgNO_3$ solution used in this study to determine urine chloride content using the Fantus method was 2.9% (0.17N). Thus, to ensure these levels, standardization was carried out using a 0.1N NaCl solution. The standardization resulted in the $AgNO_3$ level of 0.17N (2.9%), which met the expectation.

Determination of Control Material Levels with Addition and Spike Placebo Simulation

The determination of the material levels using addition control and spike placebo simulation was carried out through 30 repetitions. The determination of the levels of control materials with addition resulted in an average value of normal urine chloride control level of 197.18 mEq/L and the average value of pathologically high urine chloride control level of 309.86 mEq/L. Meanwhile, the determination of control material level using spike placebo simulation yielded an average value of normal urine chloride control level of 198.11 mEq/L and an average value of pathologically high urine chloride control levels of 313.62 mEq/L.

Performance of Homemade Control Materials with Addition and Placebo Spike Simulation

Calculation of the accuracy (%R), bias (%d), and coefficient of variation (%CV) was carried out after identifying the control levels of urine chloride in the two experimental groups. The values of accuracy (%R), bias, and precision (CV%) are used in determining the total error of the analysis (Riyanto, 2014). The accuracy value (%R) shows the closeness of the measurement result to the actual value that has been defined using the standard method. One way to assess accuracy (%R) is to calculate the recovery value. Recovery value is obtained by measuring the sample material that has been added with pure analytes, and then the average measurement result is compared with the actual value (Siregar, 2018). The recovery value was obtained using the following equation:

$$Recovery\ (\%R) = \frac{\overline{X}}{TV} \times 100\%$$

Notes:
%R = recovery percentage
TV = true value
X = mean

According to SNI (2009), a good accuracy value (%R) is in the range of 90%-110%. The acceptable bias tolerance value is <7% (CDC, 2014). To calculate the bias value (%d) in this study, the following equation was applied:

$$Bias\ (\%d) = \frac{X - TV}{TV} X\ 100\%$$

Notes:
%d = recovery percentage
TV = true value
X = mean

The measurement results of accuracy (%R) and bias (%d) of homemade control materials with addition and spike placebo simulation on urine chloride examination with the Fantus method are presented in Table 1.

Table 1. The Measurement Results of Accuracy (%R) and Bias (%d) of Homemade Control Materials with Addition and Spike Placebo Simulation on Urine Chloride Examination with the Fantus Method

Type of Control Material	Control Level	True Value (mEq/L)	Accuracy (%R)	Bias (%d)
Addition	Normal	180	109.55	9.55
	High Pathology	300	104.30	4.30
Spike Placebo Simulation	Normal	180	110.07	10.07
	High Pathology	300	104.54	4.54

Urine chloride examination using the Fantus method is a development of argentometric titration, which is applied in the clinical field. Pathologically high urine control is made at a level of 300 mEq/L based on the abnormal values of urine chloride in patients with metabolic acidosis (Panuccio et al, 2019). According to SNI (2009), in measuring chloride (Cl) using the argentometric method, a good accuracy value (%R) is in the range of 90-110%. The results of this study indicate that the accuracy values (%R) of addition and spike placebo simulation examinations at high normal and pathological levels were 109.55%; 104.30%; 110.07% and 104.54%. The values are in the range of 90-110% and considered accurate because the values were acceptable.

Chloride examination using the Fantus method was strongly influenced by observation of color changes at the equivalence point. The expected equivalence point in this reaction was the formation of a brick-red precipitate. The reactions that occurred are as follows (Ngibad & Dheasy, 2019):

$$Cl\text{-}(aq) + AgNO_3(aq) \rightarrow AgCl(s) + NO_3^-(aq)$$

$$CrO_4^{2-}(aq) + AgNO_3(aq) \rightarrow Ag_2CrO_4(aq) + NO_3^-(aq)$$

The reactions depict that the chloride ion (Cl-) reacted with $AgNO_3$ to form a white precipitate of AgCl (silver chloride). Furthermore, $AgNO_3$ (silver nitrate) reacted with CrO_4^{2-} (chromate ion) to form a brick-red precipitate of Ag_2CrO_4 (silver chromate), which was stable. The disadvantage of the Fantus method of chloride examination was that 20% K_2CrO_4 solution, with a yellow color, gave an intense color to the test solution, resulting in the formation of a brick-red precipitate that was difficult to observe. As a consequence, excess droplets during the reaction occurred. This situation was also found in the previous research by Kormaz (2017) on the chloride examinations by argentometric titration using $AgNO_3$ solution.

The results of the bias test (d%) of the control materials produced using addition and spike placebo simulation on urine chloride examination using the Fantus method in this study were 9.55%, 4.30%, 10.07%, and 4.54%, respectively. The results have proven that the urine control examination shows a good bias value, which is not higher than 7%, following the guidelines in the CDC (2014) regarding electrolytes (CDC, 2014). Perfect accuracy and bias examination results showed the values of 100% and 0%. In this study, the results of the accuracy tests on the control materials produced with addition and spike placebo simulation were not 100% accurate. Likewise, the bias value (%d) was not exactly 0%. This is because, in an inspection method, errors might occur and cause accuracy (%R) and bias (%d) values to be not 100% accurate. The errors can be both personal and systematic errors. The accuracy (%R) and bias (%d) can be used to estimate the inaccuracy of results and are assessed based on differences in the means of results (Iqbal & Tazeen, 2017). The coefficient of variation indicates the accuracy or precision of a measurement. This coefficient is a measure of the closeness of the analysis results obtained from a series of repeated measurements of the same examination. Measurement precision or accuracy can be assessed by considering the value of the coefficient of variation (%CV) in the examination data (Riyanto, 2014). The measurement results of coefficient of variation (%CV) of control materials from addition and spike placebo simulation on the urine chloride examination with the Fantus method are presented in Table 2.

Table 2. The Examination Results of Coefficient of Variation (%CV) of Control Materials from Addition and Spike Placebo Simulation

Type of Control Material	Control Level	True Value (mEq/L)	%CV
Addition	Normal	180	3.75
	High Pathology	300	3.07
Spike Placebo Simula-tion	Normal	180	4.55
	High Pathology	300	3.11

Table 2 shows that the %CV values of control materials from addition and spike-placebo simulation examination of urine chloride using the Fantus method in this study, from normal to pathologically high levels, were 3.75%; 3.07%, 4.55%, and 3.11%. The %CV values were obtained from the following equations:

$$CV(\%) = \frac{SD}{\overline{X}} \times 100\%$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}}$$

Notes:
SD : standard deviation
X_i : i^{th} data
n : number of data
$\overline{X}$: mean of examination result
CV : coefficient of variation

Standard Deviation (SD) is an illustration of the average distribution of examinations showing a value of 7.40%, while %CV or precision is a measure that represents the degree of conformity of the results measured through the distribution of individual results with procedures that are carried out repeatedly (Riyanto, 2014).

In this study, the precision or accuracy of the inspection is expressed in the coefficient of variation (%CV). The value of %VC for normal urine addition control was 3.75%. Based on the Center for Disease Control (CDC) (2014) regarding electrolytes, the CV value for chloride analytes in this study exceeded the accepted limit of 2.2% (CDC, 2014).

The results of the coefficient of variation test of control materials from addition and spike placebo simulation examination of urine chloride using the Fantus method in this study, from normal to pathologically high levels, were 3.75%; 3.07%, 4.55%, and 3.11%, respectively. The %CV values exceeded the tolerance limit set by the CDC (2014), which is 2.2%. This signifies that the distribution of data in the true value area is inadequate. This is influenced by random errors during the examination.

Random errors are influenced by instrument instability, variations in temperature or reagents, diversity of techniques, and different operators (Riyanto, 2014). Because the Fantus method of urine chloride examination is still included as a type of classical method of examination, such errors can arise when research is carried out. The distribution in the true value area, which is not good, also happens because of the ranges of droplets that cause a significant difference in the true value, which is around 28.17 mEq/L.

CONCLUSION

Based on the results of this study, it can be concluded that based on the parameters of accuracy (%R) and bias (%d), the homemade control materials from addition and spike placebo simulation have relatively good performance for the examination of urine chloride using the Fantus method. Meanwhile, based on the coefficient of variation (%CV) parameter, the homemade control materials from addition and spike placebo simulation have slightly poor performance for the urine chloride examination using the Fantus method.

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