

## Comparison of Glucose Reduction in Urine Using Benedict Method Heated by Methylated Flame with 100°C Waterbath

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### Abstract

The high prevalence of Diabetes Melitus (DM) is a global problem that must be solved by health workers around the world. This study aims to determine the differences in the results of urine reduction examination using benedict method heated by spirtus flame and waterbath 100°C. This research method is a laboratory experiment. The results of this study indicated that the urine reduction examination by heating methylated flame and waterbath 100°C shows the same results from negative (-) until positive (+4). Examination of urine reduction by heating the methylated flame and waterbath 100°C did not affect the results. However, there are a difference in the process of urine reduction examination by heating flame which was need a longer time up to 3-5 minutes. Additionally, the solution in the tube could be exploded. It was also time consuming which could only carry out one by one sample. Meanwhile, the heating of urine by using 100°C waterbath is relatively faster, which only took 2 minutes. The urine was not also exploded when it was boiled and the heating process could perform 6-8 samples at the same time (depending on the tube rack). In conclusion, the heating method of urine by using waterbath was better than spirtus (methanol) flame since it could carry out large sample in one time and it was safer for the laboratory personnel.

### Keywords

Urine reduction, glucose, benedict, methylated flame, waterbath

## INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohy-drate metabolism in which glucose is underutilized, producing hyperglycemia. Some individuals may experience acute life-threatening hyperglycemic episodes, such as ketoacidosis

or hyperosmolar coma. As the disease progresses, individuals are at increased risk for the development of specific complications, including retinopathy (which may lead to blindness), renal failure, neuropathy (nerve damage), and atherosclerosis. The last condition may result

in stroke, gangrene, or coronary artery disease (1–3).

Diabetes was initially diagnosed by the oral glucose tolerance test (OGTT). In 1979 a work group of the National Diabetes Data Group proposed modified criteria for diagnosis. This classification scheme recognized two major forms of type I diabetes (insulin-dependent) diabetes mellitus (IDDM) and type II (non-insulin-dependent) diabetes mellitus (NIDDM). To base the classification on etiology rather than treatment, in 1995 the American Diabetes Association (ADA) established a work group to reexamine the classification and diagnosis of diabetes mellitus. The revised classification, published in 1997, eliminates the terms insulin dependent diabetes mellitus and non insulin dependent diabetes mellitus, which now are termed type 1 diabetes and type 2 diabetes, respectively. Another significant change is the elimination of the categories of previous abnormality of glucose tolerance and potential abnormality of glucose tolerance (1, 2, 4).

Various sugars may be found in the urine under certain circumstances, both pathologic and physiologic condition. These include glucose, fructose, galactose, lactose, maltose, pentose and sucrose (5).

The presence of detectable amounts of glucose in urine is termed glycosuria; this condition occurs whenever the glucose level in the blood surpasses the renal tubule

capacity for reabsorption. Glucose may appear in the urine at different blood glucose levels. However, it is not usually indicated as hyperglycemia. Glomerular blood flow, tubular reabsorption rate, and urine flow will also influence its appearance. When hyperglycemia is present, however, glycosuria usually occurs when the blood level is greater than 180–200 mg/dL (1,6).

Although hyperglycemia *per se* is not necessarily indicative of diabetes mellitus, the appearance of glucose in the urine necessitates further workup. When glycosuria is present, it is typically accompanied by polyuria and thirst. Inadequate carbohydrate utilization in these patients results in elevated ketone levels in the blood and urine due to increased in fat metabolism (1).

The advantage of a urine method over a blood test for glucose is that it is painless and inexpensive for diabetic individual. Urine glucose measurements are most useful for well-controlled diabetic individuals who do not have to make frequent adjustments in their insulin/hypoglycemic agents. In insulin-dependent diabetes, a negative urine measurement could correspond to a wide range of serum glucose levels; this is attributed to the great variation in renal threshold for glucose in diabetic patients. Therefore, urine measurements may be misleading, and home blood glucose monitoring is more preferable (7).

Copper Reduction Tests (Benedict Test) as a screening test, the glucose oxidase method will not detect increased levels of galactose or other sugars in urine. It is therefore important that a copper reduction method should be used, especially for young pediatric patients. Of the copper reduction methods used for screening purposes, the qualitative Benedict method is more sensitive in reducing substances in urine than is the single-tablet (Clinitest) copper reduction method (5,8).

Urinary glucose examination using benedict method utilizes the glucose as a reducing agent. The principle of examining benedict is that glucose in the urine will reduce cuprisulfate to cuprosulfat, which can be seen by changing the color of the benedict solution. Positive results are indicated by turbidity and color changes from blue to yellowish green to brick red.

Examination of the benedict method can be done by using heating with methylated flame to boil. The weakness of this method is time consuming which can only perform one sampel per process. there is also a risk of accidents in the laboratory (1,5).

Waterbath laboratory equipment can also be used for the diagnosis of disease. For the diagnosis of samples from a patient, temperature stability is needed, so that the diagnostic results are truly appropriate. The principle of Waterbath is that when cold sterilizes the plug is turned on, the

temperature is desired and set. Arrangements must be made according to the readings of the thermostat or according to a temperature monitoring system. The advantages of waterbath are faster to get the results, 8-10 samples can be done at a once time, and the risk of accidents in the laboratory is relatively poor (9,10).

## MATERIALS AND METHODS

Benedict qualitative reagent contains cupric ion complexed to citrate in alkaline slnution. Reducing substances convert cupric to cuprous ions, forming yellow cuprous hydroxide or red cuprous oxide. The, tablets contain anhy- drous cupric sulfate, NaOH, citric acid, and sodium bicarbonate (NaHCO). Five drops (0.25 mL) of urine are mixed with 10 drops of water in a test tube. It was then mixed and immediately observed the color. A chart orovided by the manufacturer is used to the result. Heat was generated by contacting NaOH and water. The initial reaction between citric acid and NaHCO caused the release of carbon dioxide, which blankets the mixture and reduces contact with oxygen from the air to prevent reoxidation of cuprous ions. If large quantities ( $>2$  g/dL) of sugar are present in the urine, the solution goes through the range of colors and returns to greenish-brown. (8)

Material that used in this experiment was Methylated falme, waterbath, tubes, benedict reagent and timer. the experimental method

in this study used the design type One Group Pretest-Postes Design, We used 3 times repetition with tubes used Methylated fire and Waterbath 100°C to determine benedict test result by using a sampel that were known for the glucose level.

The population in this study were laboratory workers who worked in the Pelopor II Regiment Clinical Laboratory, North Bogor. The sample in this study was fresh urine of Pelopor II Troop Regiment Clinic Laboratory officers, North Bogor.

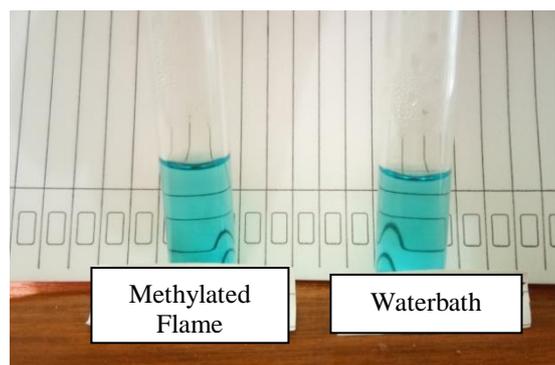
The study had begun in August 2018 until September 2018. The research was conducted at Pelopor II Regiment Clinical Laboratory, North Bogor.

## RESULTS

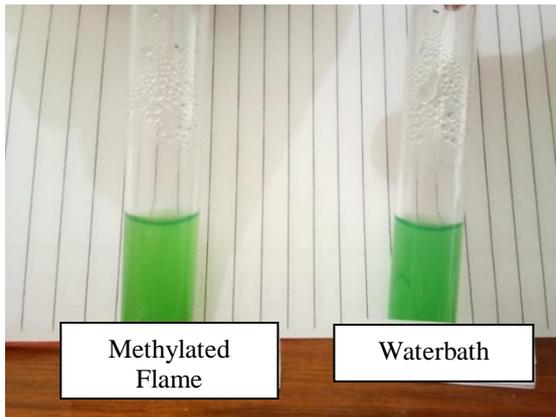
The results of the comparison study of glucose reduction in urine using heating method of methylated flame and 100°C waterbath can be seen in the following Tabel 1.

**Table 1.** Results of Benedict Tests

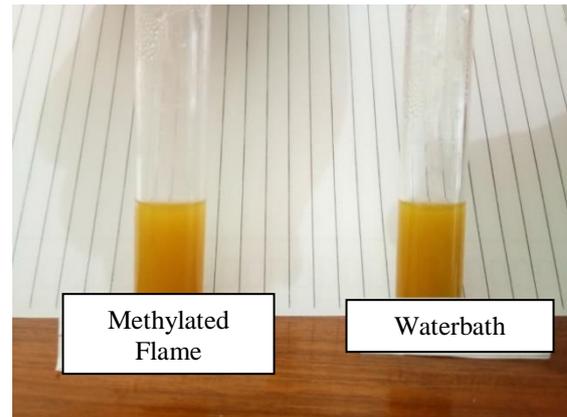
Heating Method	Result		
	Repetition 1	Repetition 2	Repetition 3
Methylated Flame	Negative (-)	Negative (-)	Negative (-)
	Positive (+1)	Positive (+1)	Positive (+1)
	Positive (+2)	Positive (+1)	Positive (+1)
	Positive (+3)	Positive (+3)	Positive (+3)
	Positive (+4)	Positive (+4)	Positive (+4)
Waterbath 100°C	Negative (-)	Negative (-)	Negative (-)
	Positive (+1)	Positive (+1)	Positive (+1)
	Positive (+2)	Positive (+1)	Positive (+1)
	Positive (+3)	Positive (+3)	Positive (+3)
	Positive (+4)	Positive (+4)	Positive (+4)



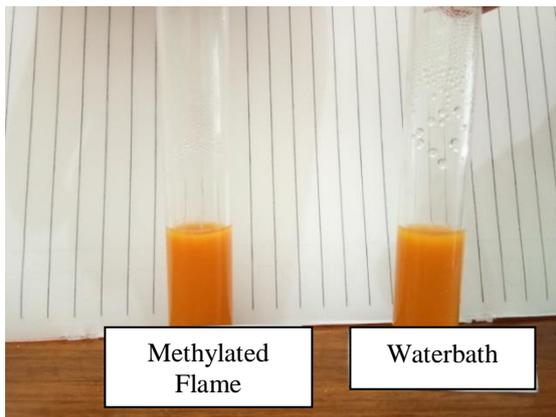
**Fig 1.** Glucose Reduction in Urine Negative Results



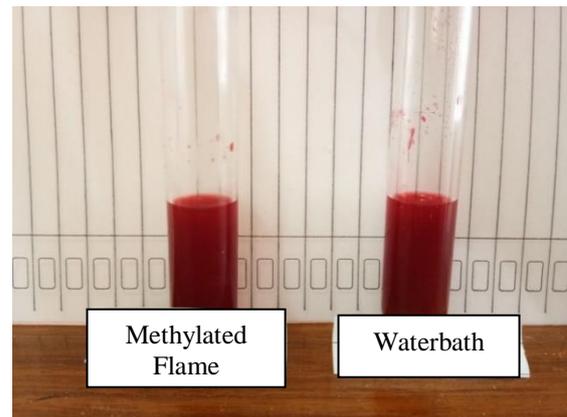
**Fig 2.** Glucose Reduction in Urine Positive (+1) Results



**Fig 3.** Glucose Reduction in Urine Positive (+2) Results



**Fig 4.** Glucose Reduction in Urine Positive (+3) Results



**Fig 5.** Glucose Reduction in Urine Positive (+4) Results

From the picture above, the examination of glucose by Benedict test heated by Methylated flame and Waterbath 100°C showed exactly same result. The number of samples used in the examination of glucose reduction in urine done by the benedict test heated by methylated flame and 100°C waterbath were 15 samples each. the total number of samples were 30 samples with 3 repetitions. The results were obtained by using urine samples that have been given additional glucose with various treatments in

accordance with predetermined values (5,11).

There result is that normal urine samples without addition of glucose showed a negative result with blue color ( there was no change in color). Meanwhile, urine samples with the addition of 0.05gr of glucose dissolved in 10mL of urine showed a positive result (+1) with green / yellowish green color. Besides, the urine samples with the addition of 0.15gr glucose dissolved in 10 mL of urine showed a positive result (+2) with cloudy yellow color. Similarly, urine samples with

the addition of 0.30g of glucose dissolved in 10 mL of urine showed a positive result (+3) with cloudy orange color. Lastly, the sample

## DISCUSSION

Sugars may be called as reducing or nonreducing based on their ability to reduce copper during the Benedict's test. The reducing property of sugar is based on the presence of free aldehyde or ketone group in them. Most of monosaccharides and disaccharides are reducing sugars, while sucrose is nonreducing sugar. Reducing sugars are capable of reducing  $\text{Cu}^{2+}$  (cupric ions) to  $\text{Cu}^+$  (cuprous ions) in alkaline medium which produces red precipitate of cuprous oxide or yellow precipitate of cuprous hydroxide. The urine of normal individuals contains small amount of reducing substances which are not sufficient to give positive test with Benedict's test or Fehling's test. Various reducing sugars present in the urine are glucose, galactose, fructose, and lactose.

Examination of urine for glucose is rapid, inexpensive, and noninvasive and is used to screen large numbers of samples. The monitoring of urine glucose lacks sensitivity and specificity and provides no information about blood glucose concentrations below the renal threshold (usually 180 mg/dL). The older screening tests detect all sugars that reduce copper and also react with reducing substances other than sugars. Specific tests

with the addition of 0.50gr of glucose dissolved in 10 mL of urine showed a positive result (+4) with red brick color (1,8,10).

for measurement of glucose that are quantitative or semi-quantitative are widely available and have essentially replaced the nonspecific tests in adults. The copper reduction test is used to screen neonates and infants for inborn errors of metabolism that may result in the appearance of reducing sugars other than glucose (e.g., galactose or fructose) in the urine (12,13).

Heating is part of the urine glucose examination with benedict method and heating only serves to help the occurrence of the reduction process so that the temperature used is boiling either with methylated fire or waterbath will not affect the results of urine reduction (1,6,14).

Both methylated or waterbath are common tools in laboratories, both of these tools have the same function which is to heat. The optimum temperature that can be achieved by both is  $100^{\circ}\text{C}$  so that basically these two tools have the same in heating, although methylated has an affordable price and easy to obtain but there are weaknesses also accompany its use, for example the performance can only be done one by one so that it slows down the performance in the laboratory.

Waterbath is the preferred source of heat for several things. Waterbath is popularly used mainly to heat flammable chemicals so

there is no need to use open fires and can prevent fires. Waterbaths are made from containers filled with hot water. All waterbaths in the laboratory are equipped with interfaces, these interfaces can be either digital or analog, this allows the user to set the desired temperature. Waterbath can also be used as a heating reagent. In addition, this tool can also be used to activate certain chemical reactions (such as the urine reduction test reaction benedict method) that can occur at high temperatures.

Heating is part of the urine glucose examination with benedict method and heating only serves to help the occurrence of the reduction process so that the temperature used is boiling either with methylated fire or waterbath will not affect the results of urine reduction (1,6,14).

## CONCLUSIONS

Examination of urine reduction by heating the methylated flame and waterbath 100°C does not affect the results. But there is a difference in the process with heating flame need a longer time is 3-5 minutes, when boiling the solution in the tube could be explodes and can only work one by one sample, while the heating of 100°C waterbath the time required is relatively faster, 2 minutes, not explode when boiling and can work 6-8 samples at the same time.

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## CONFLICT OF INTEREST

There are no conflicts of interest.

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