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Original Article

Comparison of Serum Stability Against Uric Acid and Glucose

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ABSTRACT

The selection of tools, especially the use of vacutainer tubes, affects the results of the examination to determine the stability of serum from plain vacutainer with vacutainer separation gel on the quality of uric acid and glucose test results. This study used an experimental design. The study took blood samples of 3 ml each in SST vacutainer and plain vacutainer serum. The mean uric acid levels using plain vacutainer and SST vacutainer were 4.5875 ± 0.59048 and 4.9250 ± 0.56318, respectively. The mean glucose levels using plain vacutainer and SST vacutainer were 90.64 ± 12.33 and 94.12 ± 13.30, respectively. Uric acid and glucose levels using gel-separated vacutainer were higher than uric acid levels using plain vacutainer. There was a significant difference in uric acid levels between regular vacutainers and SST vacutainers, similar to glucose levels ($p = 0.000$, $p = 0.000$). Uric acid and glucose levels using SST vacutainer were higher than uric acid levels using plain vacutainer. The following types of vacutainer separator gel and plain vacutainer affect the quality of uric acid and glucose test results.

KEYWORDS: Uric Acid Levels, Glucose levels, Pre-Analytics, Serum, Vacutainer Separator Gel, Vacutainer

INTRODUCTION

Good laboratory practice is an essential part of good manufacturing practice. It involves a number of good practices in the quality control (QC) laboratory which are to be undertaken to carry out an analysis with a defined degree of accuracy and precision[1]. Clinical laboratories are an integral part of health services. For example, specimen examination, laboratory service errors and errors that arise during the service can be detrimental to the patient[2]. The pre-analytic stage uses 61%, 25% analytical stage, and 14% post-analytic of the total error[3]. Maintaining the stability of blood analytes during sample storage is a significant issue in clinical laboratories. Typically, samples are stored in the refrigerator's door (4–8°C) for a brief amount of time or in the freezer (-20°C) for an extended period of time[4]. Numerous methods for improving the quality of pre-analytic and analytic [5], besides that laboratory performance can be determined by evaluated the human errors analysis of materials or specimens[6].

Accurate laboratory testing necessitates an understanding of the interactions between the collection tube and the blood specimen, which can affect the reliability of laboratory test results. However, due to the frequent occurrence of examination delays, blood specimen examinations shall be conducted immediately following the collection of samples[7–9]. One of the tools that must exist in the pre-analytic process is a blood collection tube (vacutainer) are used for serum collection for specific chemical tests, and serum gel tubes containing clot activators and gel separators are used for various laboratory tests[10]. Collecting samples, transporting them, and centrifuging them are also critical steps in the laboratory process. Serum Separator Tubes (SST) are observed during centrifugation, along with a gel barrier that has moved to the serum/clot interface[11].

Centrifugation at low speed for a brief period of time to obtain plasma or serum may result in insufficient separation of cellular blood components, while prolonged centrifugation at high speed may result in hemolysis and cell damage. The use of serum is preferred in clinical examination over plasma because does not use additional anticoagulants, the components contained in the serum are not disturbed in terms of activity and reaction to the results[12]. Prior to processing the sample, stability limits for each analyte and sample used as the basis for rejection must be determined[5]. The stability limit of an analyte is defined as the time point at which the analyte's percent deviation (PD%) reaches the maximum permissible error. Although many experimental studies have been conducted to determine the stability of most laboratory analytes under various conditions, there are some clinical guidelines that include general laboratory recommendations [13].

Blood collection tubes have a number of limitations, including an inability to store blood samples for an extended period of time and difficulty separating ~~blood~~ serum from red blood cells[14]. Additionally, prolonged contact between serum and blood cells can cause lisis. To address this issue, a SST was introduced that contained

silica and polymer gel for serum separation. At the tube's end, the serum separator gel acts as a stable chemical and physical barrier between serum and clotted blood. The ~~blood~~ serum separating gel significantly increases the stability of the serum, providing convenience in storage and transport[15,16]. The most critical factors affecting the serum separator gel's position in the SST tube are viscosity and density, centrifugation speed, temperature, and storage conditions. However, commercially available separation gels are expensive and have several performance limitations, including instability for certain analytes, polymer gels under extreme temperature conditions, the presence of gel parts or oily layers in serum, and absorption of certain substances and some steroid hormones into the gel[17].

Vacutainer plain is a tube that does not contain anticoagulants so that the test material is not contaminated and can affect the examination, so that the blood can clot naturally. According to the National Committee Clinical Laboratory System, the ideal clotting time is about 60 minutes. The advantage of vacutainers plain are relatively cheap and easy to obtain[18]. SST tubes contain silica particles and serum separation gel, frozen for approximately 15-30 minutes, freeze relatively quickly and produce higher serum levels[19].

Blood glucose examination is very important in clinical laboratories, especially diabetic patients to control carbohydrate intake in eating tests and oral glucose tolerance tests[20,21]. Diabetes mellitus is a metabolic disorder syndrome characterized by high blood glucose levels due to deficiency of insulin hormone secretion or when insulin resistance occurs, cells cannot effectively use the insulin produced[22–24]. It has long been known that glucose metabolism in serum in tubes filled with blood decreases over time. When the blood specimen has not been tested, the process of glycolysis can occur by the cellular components in it and can consume 5%–7% of the glucose contained in the sample every hour. The addition of fluoride as a glycolysis inhibitor can only be effectively used up to 4 hours of delay[23]. Renal function tests are commonly used in clinical practice to look for kidney disease, the most common of which include serum urea, uric acid, and creatinine[25–27]. Uric acid (UA) an important role in renal function and GFR[28], being the end product of purine catabolism[29]. Elevated UA levels are associated with the severity of hypertension, hyperuricemia, preeclampsia[30]. Therefore, this study aims to compare the stability of the use of a gel vacutainer separator with a plain vacutainer.

MATERIALS AND METHODS

Research Design

The study design was experimental. The location of this research was carried out at the Clinical Pathology Laboratory Faculty of Health Sciences, Universitas Muhammadiyah Surabaya, Indonesia. The samples were taken randomly as many as 16 responden's did four treatments on each sample, P1: use of plain vacutainer for uric acid; P2: use of SST vacutainer for uric acid. Sample examination used serum; P3: plain vacutainer for glucose; P4: SST vacutainer for glucose. Blood samples were taken as much as 3 ml using SST vacutainer and plain vacutainer. Preliminary tests were carried out by measuring the control serum 20 times and then making the Westgard rule.

Sample Preparation and Analysis

Sample criteria are not having a history of diabetes and gout, having a uricacid level of 4-7 mg/dl, and a glucose level of 80-110 mg/dl. The method of checking uric acid levels uses the Enzymatic Colorimetric method to determine uric acid through a reaction with uricas. H_2O_2 reacts under peroxidase catalysis with 3,5-dichloro-2-hydroxybenzene-sulfonic acid (DCHB) and 4-aminophenazone (PAP) togive purplish-red quinone imine dye as an indicator. While the method of checking glucose using GOD-PAP withthe principle that glucose in the sample is oxidized to form gluconic acid and hydrogen peroxide. POD catalyzeshydrogen peroxide 4-Aminoatypyrene with phenol indicator to form quinone imine and water. Data analysis useda statistical test with paired t-test.

RESULTS

Uric Acid

Measurement of uric acid levels in SST vacutainer and plain vacutainer obtained the following results, the average uric acid levels using plain vacutainer and with SST vacutainer were 4.5875 mg/dl and 4.9250 mg/dl, respectively (Table 1). Data were analyzed using a paired t-test. There was a significant difference in uric acid levels between plain vacutainer and SST vacutainer (p=0.000).

Table 1. Comparison of serum uric acid using a plain vacutainer and with a SST vacutainer

	P1	P2
Mean	4.5875	4.9250
SD	0.56318	0.59048
Min	4.1	4.7
Max	6.0	6.5
Paired test	0.00	

P1 for plain vacutainer; P2 for SST vacutainer

Figure 1 shows the comparison of serum stability against uric acid. The mean uric acid levels using a plain vacutainer and with a SST vacutainer had consistent results in which the mean uric acid levels used SST vacutainer was higher than plain vacutainer.

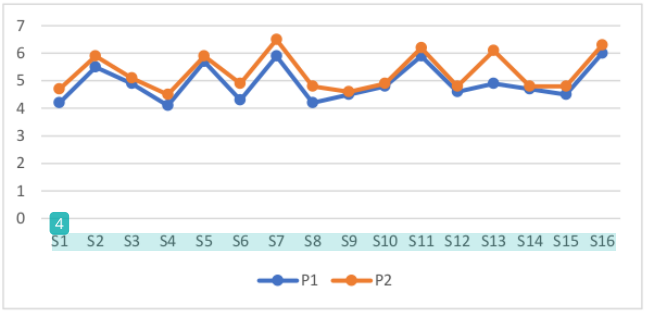

Figure 1. Comparison of serum stability against uric acid. P1 for plain vacutainer and P2 for SST vacutainer.

Glucose

Measurement of glucose levels in SST vacutainer and plain vacutainer obtained the following results, the mean glucose levels using plain vacutainer and SST vacutainer were 90.64 mg/dl and 94.12 mg/dl (Table 2). Data were analyzed using paired t-test. There was a significant difference in glucose levels between plain vacutainer and SST vacutainer ($p = 0.000$).

Tabel 2. Comparison of serum glucose using a plain vacutainer and with a SST vacutainer

	P3	P4
Mean	90.64	94,12
SD	12.33	13,30
Min	85	87
Max	98	108
Paired test	0.00	

P3 for plain vacutainer; P4 for SST vacutainer

Figure 2 shows the comparison of serum stability against glucose. The mean glucose levels using a plain vacutainer and with a SST vacutainer had consistent results in which the mean glucose levels used SST vacutainer was higher than plain vacutainer.

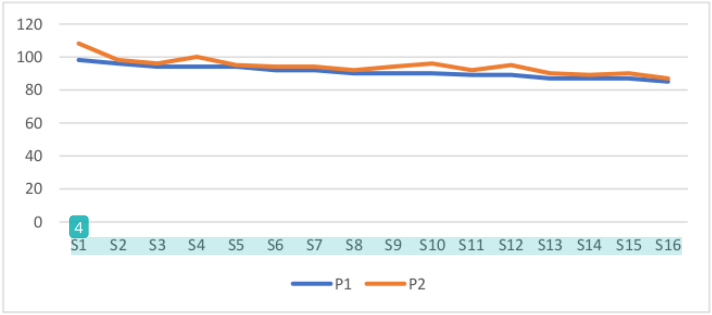

Figure 2. Comparison of serum stability against glucose. P1 for plain vacutainer and P2 for SST vacutainer.

The total processing time of the samples accommodated in the SST vacutainer was faster, with a mean of 24 minutes 51 seconds. At the same time, using a plain vacutainer blood collection tube takes a mean of 1 hour 3 minutes.

Tabel 3. Stability serum uric acid and glucose

	P1	P2	P3	P4
Mean	24':51"	1 hour:02"	24':49"	1hour;03"
Min	24':38"	59':50"	34':18"	59':58"
Max	35':38"	1 hour:05"	35':13"	1hour:06"

P1 for plain vacutainer, P2 for SST vacutainer for uric acid; P3 for plain vacutainer, P4 for SST vacutainer for glucose

DISCUSSION

This study showed that the mean values of plain vacutainer and SST vacutainer were within the normal range for uric acid and glucose levels. The mean uric acid level using SST vacutainer was higher than the mean uric acid level using plain vacutainer. These results also follow the study of urea levels conducted using three types of tubes, including serum separator tubes, lithium heparin vacutainers, and plasma separator tubes. Based on these results, the three tubes can be used as a substitute for serum for blood chemistry examination[14]. Glucose levels in this study had a higher average between SST vacutainer and plain vacutainer. This is influenced by the stability of glucose in serum to be used by cells other than blood cells, for example by the presence of microbial contamination in serum[26].

Production and manufacture for SST tubes generally have a gel-specific gravity that varies relative to each factory, as Faught's research has shown that there are differences in the specific gravity of the separating gel used in some SST tubes and between tube lots[26]. Although the SST vacutainer has the advantage of being able to optimize the workflow because of the composition of the silica clot activator and polymer gel which functions to separate serum from the clot, the results obtained are invalid or inaccurate, or inaccurate because the gel vacutainer separator also has a weakness. The gel should not be frozen because the physical composition of the gel can change so that there can be contamination between blood cells and serum, as well as instability and incompatibility of the analyte that does not match the patient sample[31]. In addition, many laboratories perform routine chemical analyzes with serum blood collection tubes containing a separating gel or polymer gel. However, previous studies have shown that gel tubes are not entirely perfect, although they have several advantages over ordinary tubes. The use of SST is generally disposable (disposable) so that it will increase the cost of purchasing the tube, especially for short-scale use at room temperature in the clinical laboratory[25].

While the advantages of SST vacutainer are workflow optimization short centrifugation time, sample processing and storage in the primary tube does not cause confusion for transfer to the secondary tube, produces better and more serum, can improve analyte stability, and reduce the rate of hemolysis during the separation process[32,33]. A drawback of the SST vacutainer is the manufacturer's stated restrictions on sample handling for SST, which contains a gel that should not be frozen, as freezing and thawing can change the physical composition of the gel, resulting in contamination of blood cells and serum. Gel instability and analyte incompatibility are caused by flotation of gel separators that are not suitable for patient samples, physical instability of polymer-based polyesters under extreme temperature conditions, and the release of lubricants that can hinder the examination process[14].

The total processing time of the samples accommodated in the SST vacutainer was faster than the plain vacutainer. The average sample processing time with a SST vacutainer is 25 minutes. At the same time, using an ordinary vacutainer blood collection tube takes an average of 1 hour 8 minutes[17]. The difference in time is because ordinary vacutainers require additional time for complete clotting on average for 35 minutes 50 seconds, while serum separator vacutainers only average about 4 minutes 38 seconds. This time calculation may differ if the work is carried out elsewhere with different room temperatures, tube sizes, and blood volumes. This is consistent with research showing that the use of gel is more profitable for laboratory technicians because it does not reduce Turn Around Time (TAT) and the presence of obstacles that can reduce sample transfer to secondary tubes and minimize the spread of potentially hazardous aerosols. Another advantage is that it can increase the stability of the analyte and reduce the rate of hemolysis during separation [31,34,35].

CONCLUSION

The uric acid and glucose level using a vacutainer separator gel was higher than the uric acid level using a plain vacutainer. The following types of vacutainer separator gel and plain vacutainer affected the quality of uric acid and glucose examination results.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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