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ABSTRACT

Blood chemistry testing requires centrifugation as one of the crucial pre-analytical phases. Usual differences in the speed and time of centrifugation influence specimens to varying extents among laboratories. This prompted the researchers to ascertain the effects of varying centrifugation speeds and times on healthy individuals' blood chemistry values and reduce the centrifugation time attested by the World Health Organization (WHO). Blood specimens were collected from four healthy adults, centrifuged, and then analyzed using the VITROS 5600 Automated Machine. The dry chemistry principle was applied. Three sets of speed and time were applied (1) 3,500 RPM for 15 minutes (WHO recommended), (2) 3,750 RPM for 10 minutes, and (3) 4,000 RPM for 5 minutes. The parameters include Fasting Blood Sugar (FBS), Total Cholesterol (Total CHOLE), Creatinine (Crea), Sodium (Na), and Potassium (K). The results of the serum chemistry values have no significant difference between different speeds and times against the standard set by WHO and were found to be acceptable, normal, and close in range with each other. Therefore, these can be substituted for the WHO-recommended speed and time. The researchers forward the following recommendations: (1) tests should be conducted to respondents with comorbidities and to younger and older individuals; (2) conduct the tests in triplicates and consider using other methods and more participants should be considered; and; (3) consider lesser time in centrifugation.

Keywords: Centrifugation, speed, time, chemistry testing, WHO recommendation

INTRODUCTION

It is widespread knowledge among healthcare professionals that one of the most crucial routines in a clinical chemistry laboratory is the process of centrifugation.

The Vector: International Journal of Emerging Science, Technology and Management
Volume 32, Issue 1, January - December 2023

It is mainly done in laboratories during the pre-analytical stage of the entire testing procedure.

A centrifuge is a device used to separate components of a liquid based on density by centrifugal force, pushing heavy parts at the bottom of the vessel or tube at high speed. A desktop-type centrifuge is commonly used to separate plasma from blood cells at various centrifugation times or durations as well as varying revolutions per minute (RPM).

Minder et al. (2011) state that laboratories in different regions primarily determine the time and speed of centrifugation they use. In Europe, blood samples are centrifuged at around 2,000 to 4,000 rpm for about two to 10 minutes at various laboratories. While some, spin at 3,500 rpm for 10 minutes. The centrifugation speed of most laboratory centrifuges is 500 to 4,000 rpm, with a centrifugation time of one to 100 minutes. A 15-minute centrifugation time is proposed by the World Health Organization (WHO) without providing references to any scientific works. This guideline is regarded as the advice of experts and suggestions from the makers of laboratory centrifugation equipment.

To acquire the best quality of a sample material, speed and time of centrifugation are critical. Prolonged centrifugation can cause hemolysis and structural damage, whereas brief, low-speed centrifugation can result in inadequate segregation of plasma or serum from the cellular blood components. As a result, chromatic interferences can affect laboratory analyses, loss of analytes being metabolized or consumed, and interference from residual cellular components.

Life is fundamentally dependent on health (Savella, 2019). Routine blood testing is one of the most crucial ways to monitor a human's overall physical health. Getting tested at routine intervals can allow one to see how their body changes over time and empower them to make informed decisions about their health (Villines & Weatherspoon, 2019). The annual medical examination uncovers several health problems, such as hypercholesterolemia, impaired fasting glucose, hyperuricemia, heart problems, hypertension, anemia, and diabetes mellitus (Tagorda et al., 2023). Hospitals and Rural Health Units/Centers assist both preventive and therapeutic medical services in society (Quilon, et al, 2019).

Jarzabek (2013) asserts that specific centrifuge parameters must be adhered to to obtain the correct analyte results in processed samples. These parameters are specified by collection tube manufacturers, centrifugation spin time, speed, and even temperature.

In today's clinical chemistry laboratory, centrifuges with specimen tubes spun at a 90° angle to the rotor shaft are the most widely used type. For 10 minutes, these devices centrifuged the bulk of tubes at 3500 rpm. All of these lapses and inconsistencies in procedure are known to have varying degrees of impact on sample quality.

Objectives of the Study

The objective of this study was to see how different durations and speeds of centrifugation would affect the values of blood chemistry tests among healthy individuals and study the possibility of reducing the time for blood centrifugation as prescribed by the WHO during the pre-analytical phase.

METHODOLOGY

Research Design

This study utilized the experimental research design. It was conducted in an actual laboratory setting. The experiment used three different variations of centrifugation speed and time, performed with five different blood tests per respondent.

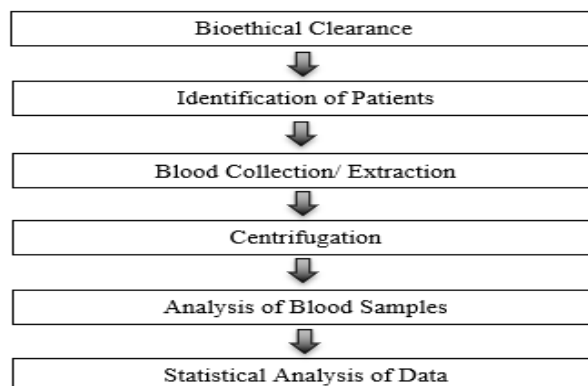
Data Gathering Procedure

The steps in the schematic flow chart are discussed in detail in this part of the study, starting from the bioethical clearance of patients to the statistical analysis of obtained data.

The diagram below illustrates the flow of the activities conducted in the study's performance. The different phases of the flowchart (Cadamuro et al., 2018) are described as follows:

Figure 1

Schematic flow chart



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I. Bioethical Clearance. The Ethics Review Committee approved the study, and the procedures conducted are within the protocols of the Committee of Human Research.

II. Identification of Patients. Four healthy adult individuals who are physically fit and have no history of any comorbidities such as diabetes, heart disease, hypertension, or substance abuse, at least two males and two females, ages 18 to 25 years old, consented to provide blood samples for the study.

III. Blood Collection/ Extraction. During the extraction of the blood samples, the respondents were required to be in a strict fasting state or NPO (nothing by mouth). Phlebotomy was performed using the syringe method. Fifteen (15) mL samples were collected from each patient.

IV. Centrifugation. A Registered Medical Technologist performed the centrifugation process. The three centrifugation conditions were as follows: 1) The first tube - 3,500 RPM for 15 minutes (WHO-prescribed), 2) the second tube - 3,750 RPM for 10 minutes for the second tube, 3) and the third tube at 4,000 RPM for 5 minutes. The VITROS 5600 Automated Machine was used, and the dry chemistry principle was applied.

V. Analysis of Blood Sample. A certified medical laboratory scientist with several years of working experience conducted all the laboratory procedures. Following centrifugation, the samples were examined. The machine and the instruments used underwent strict quality control and calibration procedures.

Data Analysis

Mean and One-way Analysis of Variance (ANOVA), were used to analyze and interpret the gathered data in this study.

RESULTS AND DISCUSSIONS

Different speed and duration of centrifugation

The serum chemistry values of healthy individuals were determined at different speeds and durations as follows: the WHO-prescribed speed and duration of 3500 RPM for 15 minutes and the experimental speed and duration of 3750 RPM for 10 minutes and 4000 RPM for 5 minutes. The table summarizes the serum chemistry values of four healthy individuals for the three speeds and durations.

Table 1

Mean serum chemistry values of the blood sample of patients centrifuged at different speeds and durations of 3,500 RPM for 15 minutes (WHO-prescribed), 3750 RPM for 10 minutes, and 4000 RPM for 5 minutes

Analytes	Centrifuged, Speed and Time		
	3500 RPM for 15 minutes (WHO- prescribed)	3750 RPM for 10 minutes (Experimental)	4000 RPM for 5 minutes (Experimental)
FBS	93	92.5	93.5
Total Chole	187.75	189.25	188.5
Crea (mg/dL)	1.175	1.175	1.15
Na (mEq/L)	139.75	140	139.75
K (mEq/L)	4.05	4.075	4.075

Considered healthy individuals, the mean blood chemistry results revealed that the respondents whose blood samples were centrifuged at the reflected speeds and time had normal blood chemistry values in terms of their FBS, Total Chole, Crea, Na, and K. It is important to note that sex and other biological factors affect the laboratory test results of a person (Center for Disease Control and Prevention (CDC), 2021).

Slight differences in the serum chemistry values of each respondent spun at varying centrifugation speed and time are observed, but such values do not pose an alarming gap among one another as they still all fall at the expected normal reference range.

The normal reference values of the analytes are the following: FBS is between 70 mg/dL (3.9 mmol/L) and 100 mg/dL (5.6 mmol/L) (Mayo Clinic, 2021); cholesterol is from 125 to 200 mg/dL (Lee, et al., 2021); Creatinine, males (0.9-1.3 mg/dL) and females (0.6-1.1 mg/dL) (University of Rochester Medical Center, 2021); and sodium is 135 to 145 milliequivalents per liter (mEq/L) (University of California San Francisco, 2019).

As seen on the table, the values obtained using the two experimental speeds and durations of 3,750 RPM for 10 minutes and 4,000 RPM for 5 minutes are close to the values gained utilizing the WHO-prescribed speed and duration of 3,500 RPM for 15 minutes.

An individual with low fasting blood glucose concentration is a hallmark of hypoglycemia. In contrast, individuals with high FBS are hyperglycemic, which suggests a low risk of diabetes. Ishii et al. (2016) mentioned that gender differences in food uptake, glycemic index, BMI, and various plasma parameters affect blood glucose levels in males and females. Females have statistically significantly lower FBS compared to males.

Blood cholesterol levels are associated with an increased risk of coronary heart disease. Total cholesterol is more than the sum of the HDL (high-density lipoprotein, or “good” cholesterol) and LDL (low-density lipoprotein, or “bad” cholesterol) numbers. Either a high HDL number or a high LDL number can make your total cholesterol number high (Bruce, 2020). Generally speaking, women have greater cholesterol levels than men because the female sex hormone estrogen increases HDL cholesterol more than men. However, it is essential to remember that everything changes during menopause (Michos, 2021).

One consequence of regular muscular activity is creatinine. It is a metabolite of creatine phosphate, which the muscles use as energy. Creatinine levels are directly related to muscle mass. For this reason, males tend to have higher creatinine levels than females (Chavoustie & Sherrell, 2021).

Potassium and sodium aid in maintaining appropriate blood pressure and supporting the work of the muscles and nerves, as well as controlling the fluid equilibrium of the body. Assessing serum sodium is a standard procedure for determining electrolyte, acid-base, and water balance and renal function. Sodium accounts for approximately 95% of the osmotically active substances in the extracellular compartment, provided that the patient is not in renal failure or does not have severe hyperglycemia (Sheehan, 2018).

Potassium intake depends on your age, gender, and specific health conditions; results may vary for these reasons. Blood potassium levels are so low that even slight variations in quantity might result in significant issues.

False potassium test results can also be a reason for varying results and may happen as the blood sample is being collected and processed. Levels of potassium may increase in collecting blood while the fist is clenched. Potassium leakage from the cells into the serum could be caused by transporting the sample to the lab or shaking it. Such factors should be considered to avoid false results and repetition of laboratory tests (Pietrangelo & Yellayi, 2020).

Allison et al. (2020) studied the effect of different centrifugation speeds and duration (within the scope of the WHO centrifugation time guidelines) on the value of

commonly measured analytes like sodium, potassium, chloride, and bicarbonate. The average values of every analyte were nearly identical for every 1000 revolutions per minute (RPM), 2000, 3000, and 4000 RPM, as well as the average values of all the samples labeled 3 minutes, 6 minutes, 9 minutes, 12 minutes, and 15 minutes. The group means differed in a statistically significant way.

Possibility of reducing the time for blood centrifugation as prescribed by the WHO during the pre-analytical phase

Table 2 shows the significant difference between and among the control and experimental groups' blood chemistry values. There are no significant differences between and among the blood chemistry values of the control (WHO-recommended speed and time of centrifugation) and experimental groups. This implies that the blood chemistry results applying the experimental speed and time and that recommended by WHO are not significantly different. Hence, the experimental speed and time may be used alternatively to reduce the centrifugation time of 15 minutes, thereby shortening the pre-analytical phase without altering the quality of the analytical results (Tantisaranon et al., 2023).

Table 2

Significant difference in the blood chemistry results processed at different centrifugal speed and time

Variables	(Differential) Df	Critical value (At 0.05)	Statistics F	Analysis
FBS Blood Chemistry Values	3/12	2.39	37.71	Not significant
Total Chole Blood Chemistry Values	3/12	2.39	44.72	Not significant
Crea Blood Chemistry Values	3/12	2.39	38.24	Not significant
Na Blood Chemistry Values	3/12	2.39	59.33	Not significant
K Blood Chemistry Values	3/12	2.39	54.77	Not significant

CONCLUSIONS

After a thorough analysis and interpretation of data, the researchers conclude that no significant difference exists between and among the blood chemistry values of the control (WHO-recommended speed and time of centrifugation) and the experimental groups. Therefore, the experimental centrifugation speed and time of 3,750 RPM for 10 minutes and 4,000 RPM for 5 minutes can be used as an alternative to the standard set by WHO at 3,500 RPM for 15 minutes to reduce the pre-analytical phase of the testing process. A decreased centrifugation time from 15 to 10 or 5 min could result in a turn-around time that is faster without significant clinical effects on routine serum chemistry tests.

RECOMMENDATIONS

Based on the conclusions drawn, the following are recommended: 1. Tests should also be conducted on participants with comorbidities to compare the results against individuals with healthy lifestyles. Likewise, they may also be conducted on younger and older individuals than the age required in the study. 2. Researchers should conduct the tests in triplicates and consider using other methods for a better basis for the study. More participants should be considered. 3. Less time in centrifugation may be considered in performing tests to avoid consuming time in the pre-analytical phase.

ETHICAL STATEMENT

This study's protocols were all carried out in compliance with the approved protocols of the Committee on Human Research. Moreover, informed consent, both orally and in writing, was collected from the patients to anonymize their information to be published in this article.

ACKNOWLEDGMENT

The researchers hope to convey their gratitude and their sincere appreciation for the assistance extended by people who provided their priceless time, ongoing inspiration, and overfilling ideas and helpful suggestions in the realization of this study, particularly to the University of Northern Philippines (UNP) President Dr. Erwin F. Cadorna, the UNP- University Research and Development Office headed by Dr. Edelyn A. Cadorna, and the College of Health Sciences-Bachelor of Science in Medical Laboratory Science.

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Volume 32, Issue 1, January - December 2023

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