IMPACT OF UNDER-FILLED K_2EDTA COATED BLOOD COLLECTION TUBE ON TEST RESULTS OF COMPLETE BLOOD COUNT, RETICULOCYTE COUNT AND WHITE BLOOD CELL DIFFERENTIAL COUNT

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

(LAAVANYA KARUNANITHI)

Date: January 2025

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(LAAVANYA KARUNANITHI)

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LIST OF SYMBOLS

%	Percentage
±	Plus/Minus
=	Equals
°	Degree
µg	Microgram
µl	Microliter
fl	Femtoliter
g/dl	Grams per deciliter
l	Liter
mg	Milligram
ml	Milliliter
mm	Millimeter
pg	Picogram
×	Multiplied by
$\times 10^{12}$	Units per 10^{12} per liter
$\times 10^{9}$	Units per 10^{9} per liter
+	Plus
−	Minus

LIST OF ABBREVIATIONS

BD	Becton, Dickinson Company
CBC	Complete blood count
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Hb	Hemoglobin
Hct	Hematocrit
HPUSM	Hospital Pakar Universiti Sains Malaysia
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
K_3EDTA	Tripotassium ethylenediaminetetraacetic acid
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MS ISO 15189	Malaysian Standard of International Organization for Standardization 15189
r	Correlation coefficient
RBC	Red blood cell
RDW	Red cell distribution width
RNA	Ribonucleic acid
SD	Standard deviation
TAT	Turnaround time
WBC	White blood cell

KESAN TIUB K_2EDTA YANG TIDAK DIISI PENUH TERHADAP KEPUTUSAN UJIAN KIRAAN DARAH LENGKAP, RETIKULOSIT DAN PEMBEZAAN SEL DARAH PUTIH

ABSTRAK

Kadar penolakan sampel darah yang tinggi merupakan isu utama yang dihadapi oleh makmal hematologi pada masa ini akibat pengumpulan sampel yang tidak mencukupi, terutamanya dalam kalangan pesakit pediatrik, geriatrik, dan onkologi di mana akses vena adalah sukar. Tiub pengumpulan darah yang tidak diisi penuh memberi kesan negatif kepada nisbah darah kepada antikoagulan, yang membawa kepada keputusan yang tidak tepat. Kajian ini menilai ketepatan dan kebolehpercayaan parameter kiraan darah lengkap, kiraan retikulosit, dan kiraan pembezaan sel darah putih daripada tiub pengumpulan darah yang tidak diisi penuh berbanding dengan tiub isipadu standard. Dalam kajian ini, tujuan adalah untuk membandingkan parameter hematologi pada pelbagai isipadu pengumpulan; 0.5 ml, 1.0 ml, 1.5 ml, dan 2.0 ml dalam tiub K_2EDTA standard penutup ungu 2.0 ml. Keputusan menunjukkan bahawa kebanyakan parameter kekal konsisten dan berada dalam julat yang boleh diterima secara klinikal pada isi padu serendah 1.5 ml. Beberapa parameter seperti sel darah merah ($x10^{12}$/l), hematokrit(%) and limfosit ($x10^9$/l) kekal stabil walaupun pada isipadu 1.0 ml. Namun, hemoglobin menunjukkan perbezaan ketara pada isipadu yang lebih rendah. Oleh itu, tiub yang tidak diisi penuh boleh digunakan sebagai alternatif, mengurangkan penolakan sampel dan menurunkan kos keseluruhan penjagaan kesihatan. Kajian masa depan harus merangkumi populasi yang lebih dengan pelbagai alat analisis yang berbeza untuk menyokong kajian ini.

ABSTRACT

High blood sample rejection rate is a prominent issue faced by hematology laboratories these days due to insufficient sample collection especially in pediatric, geriatric and oncology patients where venous access is difficult. Under-filled blood collection tubes affect the blood-to-anticoagulant ratio negatively leading to inaccurate results. This study evaluated the accuracy and reliability of complete blood count, reticulocyte count and white blood cell differential count parameters from under-filled blood collection tubes compared to standard volumes tubes. In this study, the aim is to compare hematological parameters across different collection volumes; 0.5 ml, 1.0 ml, 1.5 ml, and 2.0 ml in 2.0 ml standard lavender top K_2EDTA tubes. The results have shown that most parameters remain consistent and were within clinically acceptable ranges in volumes as low as 1.5 ml. Some parameters, such as red blood cell ($x10^{12}$/l), hematocrit (%) and lymphocyte ($x10^9$/l) remain stable even at 1.0 ml. This excludes hemoglobin, which has significant differences to all lower volumes. Therefore, under-filled tubes may be used as an alternative, reducing sample rejections and reducing overall healthcare costs. Future studies should include diverse populations and different analysers to support the findings.

CHAPTER 1 INTRODUCTION

1.1 Research background

A hematology laboratory is where tests are conducted on blood samples to provides accurate and reliable laboratory investigation results that are essential for the diagnosis and treatment of a patient (Noordin and Isa, 2021). Errors can occur at any stage of laboratory testing. These errors can be divided into three categories which are pre-analytical errors, analytical errors and post-analytical errors. In 2021, 46% - 70% of laboratory errors occur across the pre-analytical phase (Noordin and Isa, 2021). Amid various pre-analytical errors, blood collection error is the most commonly occurring error. Hematology laboratories reject blood samples that fail to meet predefined criteria for testing.

One of the reasons for rejection is under-filling of blood collection by the healthcare staff. This occurs when the blood drawn into collection tube is less than the recommended volume stated in the guidelines. According to the Clinical and Laboratory Standards Institute (CLSI) in their document “Tubes and Additives for Venous Blood Specimen Collection”, the blood volume collected should not fall more than 10% below the manufacturer’s stated draw volume (CLSI, 2010).

Although modern hematological analyzers require very little amount of blood samples, but a minimum threshold value is fundamental for testing (Lippi *et al.*, 2018). Another CLSI document (2008), "Procedures for the Handling and Processing of Blood Specimens”, states that the amount of anticoagulant in each blood collection tube is

specific to the volume of blood (CLSI, 2008). Collecting insufficient blood will disrupt the blood-to-anticoagulant ratio. Excessive additives in a blood collection tube will interfere with the normal chemical reactions, leading to unwanted blood clotting. Thus, the test might become false negative. On the other hand, overfilling the blood collection tube can result in pseudo polycythemia, and pseudo thrombocytopenia (Iqbal *et al.*, 2023). Pseudo polycythemia refers to falsely elevated red blood cell (RBC) count and other RBC indices while pseudo thrombocytopenia is the false low in platelet count. Actual parameters values can be masked which can lead to the misdiagnosis of conditions such as polycythemia vera and thrombocytosis.

When a blood sample is rejected, repeated phlebotomy is required to recollect blood for testing which can lead to inconvenience for patients. Past research indicates that the incidence of insufficient volume is particularly high in paediatric, geriatric and oncology wards, where peripheral vascular access is challenging (Getawa *et al.*, 2023). Patients are also prone to potential risks associated with the venipuncture procedure, such as hematoma and iatrogenic anemia. As for the hematology laboratory, the high rejection rate of blood samples will consequently lead to delays in turnaround time (TAT) (Dayalan *et al.*, 2020). Increased cost can also be identified as an issue of rejection of blood samples. This is because there is additional use of resources such as blood collection tubes and reagents.

This study examined the effects of different blood collection volumes using standard 2.0 ml lavender top tubes with K_2EDTA as the anticoagulant. It aims to compare parameters of complete blood count (CBC), reticulocyte count and white blood

cell (WBC) differential count across various blood volumes to determine if under-filled tubes provide reliable results similar to those of adequately filled tubes with standard volume. A minimum blood collection volume at which test results remain coherent can be established. Previous studies on under-filled K_2EDTA blood collection tubes indicate that hematological parameters remain unchanged with under-filling up to 75% of the recommended volume (Xu *et al.*, 2010). Additionally, CLSI guidelines were originally established previously based on liquid K_3EDTA. However, blood collection tubes used these days are spray-dried K_2EDTA tubes. Guidelines may not be precisely relevant due to their differences in their anticoagulant properties can influence hematological parameters and impact clinical interpretations.

1.2 Problem statement

The main problem addressed by this study is whether under-filled blood collection tubes with volumes less than the standard volume can be a viable alternative without compromising test results of CBC, reticulocyte count and WBC differential count. Insufficient blood samples are mostly collected from the paediatrics division (Iqbal *et al.*, 2023). This may be due to the difficulty in obtaining venous blood samples from the small and fragile veins of children and newborns. Challenges for the phlebotomist are heightened due to small vein size, anatomical variations, and a child's anxiety and uncooperativeness (Naik *et al.*, 2019). For newborns, over-collection of blood may lead to anemia. BD Microtainer™ tubes are designed to address such challenges but these tubes have their downsides. To address the drawbacks of microtainers, efforts are being made to reduce their use and prioritize standard lavender top tubes.

1.3 Objectives

1.3.1 Main objectives

To determine the accuracy and reliability of CBC, reticulocyte count and WBC differential count parameters from under-filled blood collection tubes compared to standard volumes tubes.

1.3.2 Specific objectives

I. To measure and compare CBC parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

II. To measure and compare reticulocyte count parameter from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

III. To measure and compare WBC differential count parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

1.4 Hypothesis

1.4.1 Null Hypothesis (H_0)

I. There is no significant difference in the CBC parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

II. There is no significant difference in the reticulocyte count parameter from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

III. There is no significant difference in the WBC differential count parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

1.4.2 Alternative Hypothesis (H_A)

I. There is significant difference in the CBC parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

II. There is significant difference in the reticulocyte count parameter from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

III. There is significant difference in the WBC differential count parameters from blood volumes of 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml.

1.5 Rationale of study

The rationale of this study was to evaluate the possibility of using a smaller blood volume in a routine laboratory setting in Hospital Pakar Universiti Sains Malaysia (HPUSM). If smaller amounts of blood were proven adequate through this research, this will reduce the amount of blood drawn from patients. For those who require frequent blood draws, such as the children and the elderly, this would be beneficial. Thus, patient discomfort and potential risks associated with blood collection can be lessen (Dayalan *et al.*, 2020). Concurrently, this study can significantly reduce the blood sample rejection rate in a hematology laboratory. The MS ISO15189 quality standard criteria for sample acceptance can still be fulfilled despite limited sample volume. A high rejection rate is directly correlated with high costs for both patients and the laboratory services system. Laboratory errors negatively effect on patient care by increasing TAT and necessitating redraws resulting in inaccurate diagnoses and treatment (Noor *et al.*, 2023). Ultimately, it compromises patient trust and jeopardise a laboratory's reputation in their diagnostic services. The further goal of this study was to reduce the need for microtainer use for vulnerable patients. The use of microtainers is less efficient than standard blood collection tubes in terms of cost and accessibility. Microtainers generated a significant bias in the results of hematological tests compared to standard lavender top tubes (Unal, 2019). Opting for standard blood collection tubes is preferable compared to using microtainers.

CHAPTER 2 LITERATURE REVIEW

2.1 Hematology laboratory and MS ISO15189 Accreditation

A hematology laboratory is a specialised laboratory where blood, bone marrow and body fluids are analysed by medical laboratory technologists and hematopathologist. The results reported by the laboratory can confirm clinical impressions, establish a diagnosis, screen for undetected disorders or monitor the effects of ongoing therapy on a patient (Keohane *et al.*, 2020). Common conditions diagnosed in a hematology laboratory include anemia, leukaemia, clotting disorders, and infections.

The Hematology Department and Transfusion Medicine Unit of HPUSM began operations on August 1, 1983, providing diagnostic, therapeutic, research, and educational services. It offers 45 types of diagnostic hematology tests, including 11 routine tests without appointments, 10 specialised tests without appointments, and 24 specialised tests by appointment (Hematology Department and Transfusion Medicine Unit, 2024).

The Hematology Laboratory at HPUSM has been recognised by the Skim Akreditasi Makmal Malaysia (SAMM) for complying with the MS ISO 15189 certification standard, equivalent to MS ISO/IEC 17025:2001 (Registration Number: 480), since November 1, 2010 (Hematology Department and Transfusion Medicine Unit, 2024). MS ISO15189:2014 assesses the competency of a laboratory in consistently delivering valid, reliable and accurate results. This accreditation indicates

that the tests conducted in this laboratory are of high quality and meet international standards. A total of 18 tests have been accredited under the MS ISO 15189 standard including full blood count (FBC) and erythrocyte sedimentation rate (ESR).

2.1.1 Phases of the total testing process and quality control

The total testing process is divided into three phases which are the pre-analytical, analytical, and post-analytical. Pre-analytical phase begins from the moment there is a clinical request for a laboratory test to the sample preparation for analysis. Pre-analytical errors are often associated with sample collection, handling, and transportation (Iqbal *et al.*, 2023). Common errors include incorrect patient identification, which can occur because of mislabeling or mixing up patient samples. Another pre-analytical error is incorrect sample collection, which might include using inappropriate containers and anticoagulants. Furthermore, insufficient samples are also one of the pre-analytical issue that can affect the total testing process which may result in sample rejection (Abdollahi *et al.*, 2014).

The analytical phase comprises of the testing and analysis of blood samples. Errors of this phase include instrument calibration and maintenance issues, where improper calibration or poor equipment upkeep can lead to false readings. Other than that, blood interference, such as lipids or hemoglobin (Hb) might alter the analysis procedure (Hawkins, 2012). On the other hand, the post-analytical phase involves interpretation, reporting, and communication of results. Errors in this phase include improper data entry, delay in reporting critical values and misinterpretation of results.

Quality control in the laboratory is crucial to ensure that test results are accurate, reliable, and clinically meaningful. Quality control measures must be implemented in each phase to prevent errors that can affect the validity and reliability of test results. With the help of automation, errors occurring in the analytical and post-analytical phases have significantly reduced but the errors in the preanalytical phase still occur as it is mostly dependent on manual labor (Iqbal *et al.*, 2023).

2.1.2 Rejection criteria for blood samples

A laboratory has to maintain the integrity of sample and accuracy of results, therefore they have the right to reject a sample before analysis if it does not meet the predefined criteria (Shoaib *et al.*, 2020). When samples are rejected, authorised personnel should be informed and another sample should be requested. Sample rejection may lead to a median lag of 65 minutes in TAT of test results potentially postponing diagnoses and treatment (Rooper *et al.*, 2016).

The main reason for sample rejection is insufficient collection of blood by the paramedical workers (Getawa *et al.,*2022). The rejection rate is high for pediatric, geriatric, oncology and intensive care patients due to difficulty in localising veins. Under-filled tubes have an inappropriate blood-to-anticoagulant ratio, which affects blood cell morphology. Ultimately, it is better to reject insufficient samples to avoid inaccurate and unreliable test results. Previous study has also shown that clotted blood samples were the most frequent reason for rejection in a hematology laboratory (Lay *et al.,* 2014). This occurs due to poor phlebotomy technique and improper mixing of anticoagulant with blood immediately after collection.

Furthermore, incomplete or improper labelling of specimen plays a significant role in blood sample rejection. Potential occurrence of preanalytical errors may happen because of specimen misidentification (Musa and Ndakotsu, 2020). Information such as patient's names and identification number should be reflected on specimen tube and must be matched with the tests request forms. Moreover, hemolysed samples are also rejected as they can cause falsely elevated levels of intracellular content such as Hb (Azman *et al.*, 2019). Some of the factors causing hemolysis of blood samples are vigorous shaking of tubes and forcing of blood through fine needles (Shoaib *et al.*, 2020).

2.1.3 Causes and adverse effect of insufficient sample

Among the various pre-analytical errors, the collection of an insufficient volume of a sample is a frequent occurrence with a percentage of 10 in most clinical laboratories (Benati *et al.*, 2024). In the hematology laboratory of HPUSM, blood samples with volume less than 2.0 ml will be strictly rejected. These insufficient samples are inadequate and do not meet the criteria for accurate testing. There are several causes of insufficient sample collection such as incomplete filling of tubes during blood sampling. For example, a phlebotomist may remove the tube too quickly which leads to blood spillage (World Health Organization, 2010).

Under-filling the blood collection tube results a higher ratio of anticoagulant to blood sample. Accurate ratio between anticoagulant and blood is important in a routine hematology testing. An imbalance in the ratio can lead to alteration of blood

components. In this scenario, the RBCs can potentially shrink due to increased ion concentration (Marsudi *et al.*, 2024). Abnormal or unclear RBC morphology, decreased hematocrit (Hct) values, increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) and platelet disintegration are several effects of plasma hypertonicity due to excess ethylenediaminetetraacetic acid (EDTA) (Riba *et al.*, 2020). Furthermore, smaller volumes have higher surface area to total volume ratio which increases the risk of evaporation (Benati *et al.*, 2024). Another adverse effect of insufficient sample is that it may not provide enough blood for multiple or repeated analyses, ultimately limiting laboratory's diagnostic capacity. Low volumes can also disrupt the modern analyzers performance leading to invalid test results (Benati *et al.*, 2024).

2.1.4 Clinical implications of frequent rejection of insufficient sample

Frequent rejection of insufficient sample volumes often requires repeated phlebotomy. Phlebotomy, also known as bloodletting is a procedure of drawing blood from an individual with the aid of a needle (Srikanth and Lotfollahzadeh, 2023). It is the most common invasive procedure done by a physician, a nurse or a certified phlebotomist in a health care setting.

A variety of adverse complications may be encountered by patients during blood drawing procedures. Poor practice or anatomical abnormality can cause adverse effects such as bruising at the site of pierce, nerve damage and hematoma (Ahmad *et al.*, 2023). Aside from that, vasovagal reactions may also occur. Syncope and fainting are part of autonomic nervous system reaction triggered by fear and anxiety leading to sudden fall

in blood pressure (World Health Organization, 2010). Localised infection is also one of the serious challenges of phlebotomy. Inadequate cleansing or poor aseptic technique can lead to infection such as phlebitis and thrombophlebitis (Buowari, 2013). Venipuncture may also pose potential risk to the healthcare staff performing the procedure. They are prone to needle stick injury and transmission of blood-borne diseases such as Hepatitis B, Hepatitis C and Malaria.

Rejected samples may cause a delay in analysing and processing test results. Increase in TAT leads to prolonged waiting time for diagnosis. Time is lost in informing the ward about the sample rejection, arranging for a new blood draw and processing the subsequent sample. In urgent medical cases such as bleeding disorders, sepsis and acute myocardial infarction, the delay in diagnosis may significantly affect the course of treatment and management (Car *et al.*, 2016). This might worsen patient outcomes or lead to further disease progression. Delays may extend hospital stays or require the patients to return to healthcare facility for additional tests (Politi *et al*., 2022). This process adds additional financial burden on both healthcare facility and patient. Wasted tubes and syringes, added staff time for repeated procedure and extra laboratory resources increase overall costs for hospitals (Shaik *et al.*, 2023). On the other hand, patients may spend extra for extended hospital stays, fees for repeated sample collection and potential lost wages from extended time off work (Pisu and Martin, 2022).

2.2 Routine hematology tests

Routine hematology tests are commonly used to diagnose anemia, certain cancers of the blood, infections, clotting disorders and inflammatory diseases (Johns

Hopkins Medicine, 2019). These tests include CBC, Prothrombin time (PT), activated Partial Thromboplastin Time (aPTT) and International normalised ratio (INR). Frequent rejection of hematology test results will negatively affect patient care management. When samples are rejected, the patients are subjected to repeated specimen collection, resulting in inconvenience and the discomfort of repeated phlebotomy (Karcher and Lehman, 2014). Patients' perception of quality of care is based on the timely access to and discharge from the health care facility, safety, efficient care coordination, and the number of specimens collected (Karaca and Durna, 2019).

2.2.1 Complete blood count

Complete blood count (CBC) is a test that measures the cells that makeup blood's composition. The cell components include RBC, WBC, and platelets (Brihi and Pathak, 2024). CBC test is part of routine hematology test since it is inexpensive and simple to interpret. The parameters of CBC comprises of WBC, RBC, Hb, Hct, MCH, MCHC, MCV and platelet count. Electrical impedance and light scattering are the two fundamental technologies used in CBC measurement. Parameters such as Hb, MCV, and RBC can be directly measured by electric impedance whereas parameters such as MCH, MCHC, and Hct are derived from calculations of other measured values (Yenilmez and Tuli, 2018). CBC is one of the most common laboratory tests which provides information about blood cells production and identifies an individual's oxygen-carrying capacity through the evaluation of RBC indices, Hb, and Hct which assist in diagnosing an abnormal condition of patients (Doig and Zhang, 2017).

Hb is the protein contained in RBC that is responsible for delivery of oxygen to the tissues (Barshtein *et al.*, 2024). The amount of Hb in whole blood is expressed in grams per deciliter (g/dl). According to the World Health Organization (WHO), normal references for Hb levels are 13 to 18 g/dL in adult men and 12 to 16 g/dL in adult women who are not pregnant (World Health Organization, 2010). The low level of Hb is indicated as anemia whereas high level of Hb is indicated as erythrocytosis. The causes of anemia may be due to excessive blood loss, blood cell destruction and deficient or defective production of blood cells (Brihi and Pathak, 2024). Erythrocytosis may occur because of hemoconcentration, dehydration or increased production of RBC. Hct is the percentage volume of RBC in the blood. Normal Hct levels range from 40% to 54% for men and from 36% to 48% for women (Khan *et al.*, 2013). Similarly to Hb, Hct is reduced in anemia, increased in erythrocytosis, and is influenced by changes in plasma volume.

RBC count is the amount of red cells present per unit volume of blood. The normal reference range is usually 4.5 - 5.9 $\times 10^{12}$/l in adult men and 3.9 - 5.2 $\times 10^{12}$/l in adult women (Brihi and Pathak, 2024). The other red cell indices are calculated parameters such as RDW, MCV, MCH, and MCHC. The RDW represents the degree of size variation, also known as anisocytosis in the erythrocyte population (Danese *et al.*, 2015). As the size range between cells increases, the RDW rises. MCV is defined as the average volume of the RBC present (Brihi and Pathak, 2024). MCV is commonly used to classify anemias as microcytic, normocytic, or macrocytic which indicates low MCV, normal MCV and high MCV respectively. MCH quantifies the amount of Hb per RBC and is expressed as picograms per cell. It is dependent on cell volume and

correlates closely with MCV. On the other hand, MCHC is the mean Hb concentration per unit volume of RBC, expressed in grams per deciliter. Low, normal and high MCHC signifies hypochromia, normochromia and hyperchromia respectively (Doig and Zhang, 2017).

Absolute WBC count is the number of WBC present per blood litre with normal reference interval of 4.0 - 11.0 × 10^9/l in adults (Brihi and Pathak, 2024). Elevated WBC count, also known as leukocytosis may be caused by an inflammatory stressor or myeloproliferative pathology. In contrast, low WBC count is known as leukopenia and may be due to reduced production or increased utilisation and destruction of white blood cells. The platelet count is the number of platelets per unit volume of blood expressed in cells per microliter of blood or cubic millimetre. The normal reference interval in adults is 150 - 400 ×10^9/l (Daly, 2011). A decrease in the platelet count implies thrombocytopenia whereas an increase in the platelet count implies thrombocytosis.

2.2.2 Reticulocyte count

Reticulocytes are immature RBCs produced in the bone marrow and released into the peripheral blood. An increase or decrease in reticulocyte count can be a sign of erythropoiesis activity impairment, specifically in relation to anemias and bone marrow dysfunction (Rai *et al.*, 2023). The normal reticulocyte count ranges between 0.5 % to 2.5% in adults and 2% to 6% in infants. Its normal fraction in the blood is low because there is a homeostasis between destruction of aged abnormal RBC and a low level of marrow activity required to maintain normal Hb levels (Gaur and Sehgal, 2021).

Accurate reticulocyte enumeration is critical for the diagnosis of many hematological diseases and for the grading of severity of anemia.

Immature reticulocyte fraction (IRF) is the ratio of immature reticulocytes to the total amount of reticulocytes. It represents the proportion of young reticulocytes with the highest RNA content (Morkis *et al.*, 2016). An elevated proportion of immature reticulocytes is an early indicator of increased erythropoietic response in the bone marrow. The causes of increased reticulocyte count include hemolytic anemias and acute or chronic blood loss. This condition is known as reticulocytosis. Conversely, a decrease in reticulocyte count can be caused by hypochromic anemias, aplastic anemias and myelodysplastic syndromes (Ishii and Young, 2015). This condition is known as reticulocytopenia.

2.2.3 White blood cell differential count

White blood cell (WBC), also known as leukocytes are heterogeneous group of nucleated cells which are part of the immune system and participate in innate and humoral immune responses (Tigner *et al.*, 2023). WBC can be grouped as granulocytes and agranulocytes based on the presence and absence of microscopic granules in their cytoplasm. Neutrophils, basophils, and eosinophils are part of granulocytes. These cells have azurophilic granules and other specific granules that contain substances unique to each cell's function. Agranulocytes consist of lymphocytes and monocytes. They lack both azurophilic granules and other specific granules (Tigner *et al.*, 2023).

A WBC differential count is fundamental to specify the type of WBC abnormality. This provides the relative percentage of each type of WBC and helps to reveal abnormal white blood cell populations such blasts and immature granulocytes in the peripheral blood. Leukocyte disorders can be categorised as quantitative and qualitative. In quantitative alterations, cells appear normal but are present in abnormal quantities, either in excess or deficit of normal values (Blumenreich, 2011). Alternatively, abnormal appearing cells or extrinsic cells found in the circulation are part of qualitative disorders.

Neutrophils are the most abundant type of WBC which makes up 40% to 60% of the total WBC count. The absolute neutrophil count is 1.8 - 7.8 x 10^9/l. An increase and decrease in the neutrophil count are referred to as neutrophilia and neutropenia respectively. Neutrophilia can be caused by infection and trauma while neutropenia may be due to bone marrow failure (Brihi and Pathak, 2024). Lymphocytes are the next abundant type of WBC ranging from 1.0 - 4.5 x 10^9/l. Lymphocytosis occurs when there is an increase in the lymphocyte count while lymphocytopenia occurs when there is a decrease in the lymphocyte count (Hamad and Mangla, 2020).

The other three WBC differential components are monocytes, eosinophils and basophils ranging from 0.2 - 1.0 x 10^9/l, 0.04 - 0.4 x 10^9/l and 0.01 - 0.1 x 10^9/l respectively. Monocytosis is an increase in the monocyte count while monocytopenia is a decrease in the monocyte count. Similarly, an increase in the eosinophil count is known as eosinophilia while a decrease in the eosinophil count is eosinopenia. Lastly, a

decrease in the basophil count implies basopenia whereas an increase in the basophil count implies basophilia.

2.2.4 Effect on under-filled tubes on routine hematology tests

Under-filled tubes have excess anticoagulant concentration which can cause RBC shrinkage. This leads to alteration of parameters such as MCV and Hct in CBC (Ross and Dickinson, 2021). Hemolysis due to imbalance of anticoagulant can also reduce RBC counts and Hb levels. Underestimation or overestimation of RBC indices may lead to misdiagnosis of anemia or polycythemia. Excess anticoagulant may also cause platelet clumping or aggregation (Dayalan *et al.*, 2020). Falsely low platelet count can result it misdiagnosis of bleeding or thrombotic disorders.

Under-filled tubes may impair the staining of reticulocytes that might trigger the underestimation of reticulocyte count. Misleading counts can mask the recovery phases in anemia management or delay the diagnosis of ineffective erythropoiesis. Abnormal anticoagulant ratio might induce the formation of artifacts or deteriotation of cells leading to inaccurate bone marrow suppression (Pan *et al.*, 2022). This can ultimately affect the monitoring of bone marrow activity.

As for WBC differential count, under-filled tubes might cause morphological distortion of WBCs. This can cause falsely abnormal WBC count leading to inaccurate diagnosis of leukocytosis or leukopenia. Missed identification of pathological cells such as blasts or immature granulocytes due to morphological changes might delay diagnosis of critical conditions such as acute leukaemia (Chennamadhavuni *et al.*, 2023).

2.3 Blood collection tubes

Lavender cap tubes are also known as EDTA tubes as they use ethylene-diamine-tetra-acetic acid as an anticoagulant. EDTA tubes are generally used for hematological assays (Bayot and Tadi, 2023). Anticoagulation tubes are preferred when the test requires plasma instead of serum. Types of tests that require the need of EDTA tubes are whole blood hematology determination, immunohematology testing and blood donor screening.

There are two types of K_2EDTA tubes which are vacutainers and microtainers. The blood collection tubes are made of clear latex-free polyethylene terephthalate (PET) which is shatter-resistant and a safer alternative to glass. The hemogard closure has two parts which are the inner stopper and the outer protective cap. This mechanism minimise the risk of blood splattering upon opening of the tube. It also protects the lab personnel from contact with blood on the stopper or around the outer rim of the tube (Medical, 2021).

Vacutainer tubes (Figure 2.1) are tubes that can hold about 2-10 ml of suction volume (Medical, 2021). K_2EDTA tubes are available in different volumes, including 2 ml, 3 ml, 4 ml, 6 ml, and 10 ml. It allows for efficient and easy processing of whole blood in smaller volumes as compared to whole blood bags (CGT GLOBAL, 2024). This flexibility can satisfy different diagnostic needs for different patient population.

Figure 2.1: BD Vacutainer™ K_2EDTA Tube with Lavender Hemogard Closure (CP Lab Safety, 2024)

On the other hand, microtainer tubes are small volume blood collection tubes produced in the late 1970s for the collection, transport and processing of capillary or venous blood from skin puncture (Bozdemir, 2023). Microtainer tubes are smaller in size compared to vacutainer tubes (Figure 2.2) and can hold 0.25 to 0.5 ml of blood. It is specifically designed for cases requiring minimal blood samples.

Figure 2.2: BD Microtainer™ K_2EDTA Tube with Lavender Microgard Closure (Mercedes Scientific, 2024)

Microtainer tubes can minimise the amount of blood that needs to be drawn per time. It is ideally used for pediatric, geriatric and oncology patients who have difficulty

in collecting blood samples and require frequent blood sampling (Bozdemir, 2023). Pediatric patients have difficult venous access while geriatric patients have fragile veins or collapsed veins, making phlebotomy harder.

However, there are several drawbacks of microtainer tubes. Firstly, most of the CBC analysers in use nowadays are not adapted to automated sampling of these tubes (Park *et al.*, 2011). Samples are required to be handled and loaded manually by laboratory staff. Moreover, since these tubes are smaller in size, there is no enough space for labelling patient's information. This prevents automated barcode reading and increases the risk of possible clerical error. These issues may lead to delaying of the TAT and over workload (Bozdemir, 2023). The tubes do not have pierceable caps like the larger collection tubes. Health care staff have to pierce through the hard plastic tops when filling the tubes which can pose safety concerns in regard to needle stick injuries. In addition, microtainers are six to seven times more expensive than standard blood collection tubes (Xu *et al.*, 2010). Scenarios where clotted samples have been observed more frequently in microtainers compared to vacutainers have limited the use of microtainers in laboratory medicine (Unal, 2019).

2.4 Anticoagulants in blood collection tubes

Dipotassium ethylenediaminetetraacetate also known as Edetate dipotassium is a polyamino carboxylic acid (Mohammadi *et al.*, 2013). It consists of two potassium (K^+) ions bound to the EDTA molecule. EDTA has four carboxyl groups and two amine

groups that act as chelators for divalent cations like calcium (Ca^{2+}). Calcium chelating agents bind to and sequester calcium ions.

There are two types of anticoagulants used in blood collection tubes which are K_2EDTA and K_3EDTA. K_2EDTA is the "anticoagulant of choice in specimen collection and blood cell counting" according to both CLSI and the International Council for Standardisation in Haematology (ICSH) (CLSI, 2010). It is the most common anticoagulant used in lavender top blood collection tubes. Other anticoagulants such as sodium citrate and heparin anticoagulants can still be used as alternatives for testing (Putra and Hernaningsih, 2022). K_2EDTA is usually present in liquid form where it is spray coated onto the interior surface of the tubes.

K_2EDTA effectively prevents blood clotting by binding to calcium ions present in blood (Reddel, 2015). Calcium is fundamental for a wide range of enzyme reactions of the coagulation cascade (Banfi *et al.*, 2007). By removing it, blood clotting is prevented irreversibly within the collection tube. K_2EDTA is also known to have minimal effect on blood cells where it preserves the cell morphology better than some other anticoagulant.

K_3EDTA is a tripotassium salt of ethylenediaminetetraacetic acid. It consists of three potassium ions bound to the EDTA molecule. Like K_2EDTA, K_3EDTA has four carboxyl groups and two amine groups that chelate metal ions, especially calcium. K_3EDTA vacutainer tubes typically use the anticoagulant in liquid form. Certain laboratories prefer K_3EDTA due to its consistency in liquid form where it is easier to

homogenise blood sample effectively (Marsudi *et al.*, 2024). K_2EDTA has a slower dissolution since it is in dry form. Blood samples with K_2EDTA can last up to 4 hours if there is any delay in analysis (Syuhada *et al.,* 2022). In contrast, K_3EDTA provides only short-term stability and is less effective than K_2EDTA in preserving the integrity of blood cells.

All types of EDTA anticoagulants have hyperosmolar characteristics. This causes intracellular fluid to move out, leading to shrinkage of erthyrocytes. This can influence some parameters of CBC such as Hct and MCV but these effects are mainly seen in the use of K_3EDTA (Zahraini *et al.*, 2021). This is because K_3EDTA is in liquid-form where high concentration causes an increase in the ionic concentration thus making the plasma hypertonic (Riba et al., 2020). K_3EDTA might also alter morphology of WBC and platelets by causing membrane damage. Another drawback of K_3EDTA is that as it is a liquid anticoagulant, it results in the dilution of the blood specimen (Marsudi et al., 2024).

CHAPTER 3 MATERIALS AND METHODOLOGY

3.1 Introduction

This study aimed at evaluating the impact of under-filled K_2EDTA-coated blood collection tubes on CBC, reticulocyte count, and WBC differential count. Briefly, 5 ml of blood samples were collected from 49 volunteers and immediately divided into four K_2EDTA 2.0 ml test tubes with varying volumes which were 0.5 ml, 1.0 ml, 1.5 ml, and 2.0 ml (Figure 3.1). These samples were analysed using the Mindray BC-6200 analyser, and the results are analysed statistically to identify potential deviations caused across different blood volumes.

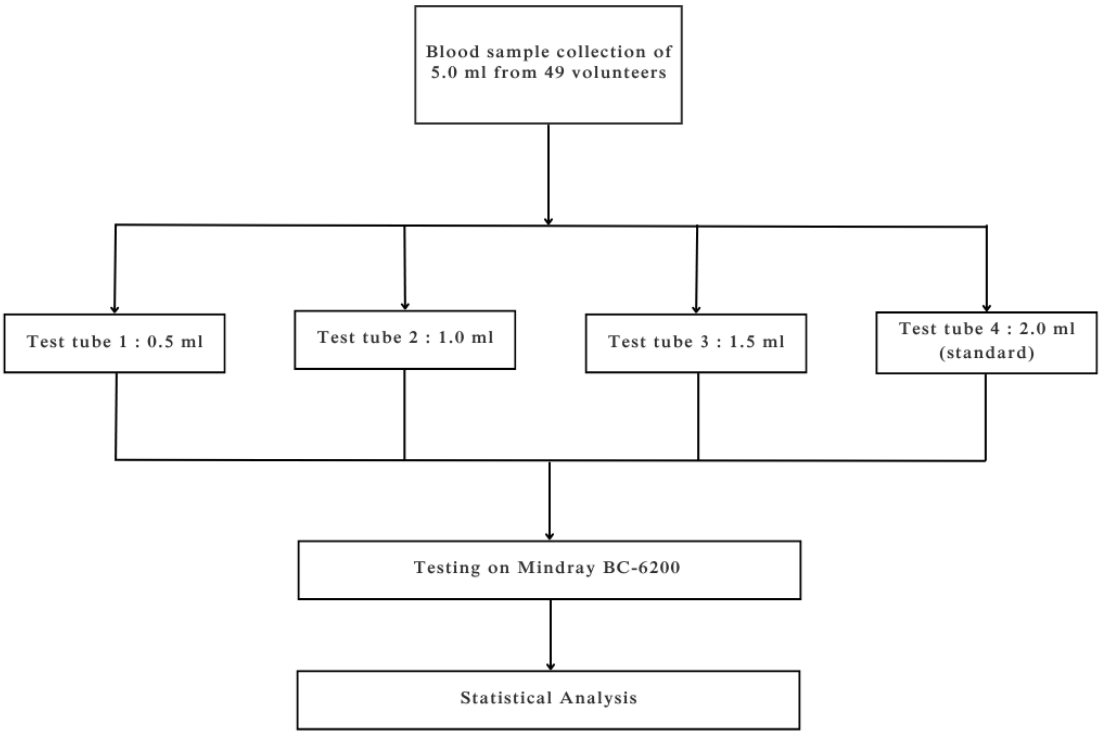

Figure 3.1: Flow chart of study on the impact of under-filled K_2EDTA coated blood collection tube on CBC, reticulocyte count and WBC differential count results