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# Estimating hematocrit using a blood drop sinking time. Siriraj Hospital, Bangkok.

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**Background** : *This is a study of correlation between a blood hematocrit value and its sinking time in oil to aid doctors in measuring a hematocrit value without the presence of a centrifuge.*

**Objective** : *The objective of this project was to provide an alternative way of measuring a hematocrit value. This method should only include materials that can be easily found in our surroundings. Measuring hematocrit is a crucial process that can help diagnose potentially fatal diseases such as dengue hemorrhagic fever.*

**Design** : *Experimental study*

**Setting** : *Siriraj Hospital, Bangkok*

**Materials and Methods** : *First, we used three different salt solutions (NaCl with specific gravity of 1.03, 1.04, and 1.05) and dropped them into the refined palm oil to see if there were visible differences in the dropping times. After confirming the validity of the experiment, we proceeded to real samples of blood. We used 23 different blood samples in two separate experiments, varying in volumes (10 μL and 20 μL).*

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**Results** : *After graphing the data acquired from the experiments, we found that there was a distinct relationship between the two values. The higher the hematocrit value, the less time it took to drop down. In contrast, the lower the hematocrit value, the slower it took to drop down.*

**Conclusion** : *The study found that there was a inverse relationship between the hematocrit value and the sinking time of blood. These graphs, tables, and data can be developed to aid doctors in obtaining a hematocrit value in places where a centrifuge is not readily available.*

**Keywords** : *Hematocrit, specific gravity.*

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**ภูมิภักดิ์ เอื้อวรากุล, อรทัย พรมสุวิชา. การวัดค่าฮีมาโตคริทโดยการใช้เวลาในการตกของหยดเลือด โรงพยาบาลศิริราช กรุงเทพมหานคร. จุฬาลงกรณ์เวชสาร 2557 ม.ค. – ก.พ.; 58(1): 27 – 34**

**เหตุผลของการทำวิจัย** : *การศึกษาความสัมพันธ์ของค่าฮีมาโตคริทกับเวลาที่เลือดใช้ในการตกของหยดเลือด ซึ่งนำมาพัฒนามาใช้ในสถานการณ์ที่ไม่มีอุปกรณ์หมุนแกว่ง (centrifuge) ที่ใช้หาค่าฮีมาโตคริท*

**วัตถุประสงค์** : *เพื่อเสนอวิธีการวัดค่าฮีมาโตคริทในแบบที่ไม่ใช่วิธีปกติ วิธีนี้ควรต้องเป็นวิธี ที่ หาอุปกรณ์ได้ตามชีวิตประจำวัน หรือหาซื้อได้ง่าย การวัดค่าฮีมาโตคริทเป็นสิ่ง ที่สำคัญต่อการวินิจฉัย และรักษาโรคเป็นอย่างมาก โรคที่สามารถใช้วิธีนี้ได้คือ โรคไข้เลือดออก*

**รูปแบบการวิจัย** : การทำงานวิจัยในห้องปฏิบัติการ

**สถานที่ทำการศึกษา** : *โรงพยาบาลศิริราช กรุงเทพมหานคร*

**ตัวอย่างและวิธีการศึกษา** : *ใช้สารละลายเกลือ (NaCl) ที่ความเข้มข้นต่างกัน (1.03, 1.04, 1.05) เพื่อวัดความต่างในเวลาของการตกในน้ำมันปาล์ม เมื่อเห็นว่ามีความต่างของเวลา อย่างชัดเจน จึงได้ทำการทดลองกับตัวอย่างเลือด 23 ตัวอย่าง และทำการทดลอง 2 ครั้งโดยใช้ปริมาตรของเลือดต่างกัน (10 $\mu$l, 20 $\mu$l) โดยได้มีค่าฮีมาโตคริทของตัวอย่างเลือดมาเปรียบเทียบ*

**ผลการศึกษา** : *พบว่าความสัมพันธ์ของค่าฮีมาโตคริทของเลือดสามารถเห็นได้อย่างชัดเจน จากกราฟที่พลอตจากข้อมูลที่ได้เก็บมาจะเห็นได้ว่าเมื่อค่าฮีมาโคตริทของเลือดมากขึ้น หยดเลือดนั้นจะใช้เวลาในการตกในน้ำมันน้อยลงในทางตรงกันข้ามกัน ถ้าค่าฮีมาโตคริทของเลือดน้อยเวลาในการตกก็จะมากขึ้น*

**สรุป** : *การศึกษาครั้งนี้ได้ผลสรุปว่าค่าฮีมาโตคริทนั้นแปรผกผันกับเวลาที่ใช้ในการตก และแปรผันตรงกับอัตราการตก ข้อมูลที่อยู่ในรูปกราฟและตารางนี้ สามารถนำไปใช้พัฒนาเป็นตารางข้อมูลที่แพทย์ใช้วัดค่าฮีมาโตคริทของเลือดโดยไม่ต้องใช้อุปกรณ์หมุนแกว่ง (centrifuge) ได้*

**คำสำคัญ** : *ฮีมาโตคริท, ความถ่วงจำเพาะ.*

Hematocrit is a common hematological parameter that is widely used in medical care. It can help diagnose and monitor many diseases or medical conditions. It is essential to patient care in hospitals and clinics. For many medical conditions such as blood loss and internal bleeding, dengue hemorrhagic fever (DHF), hematocrit can be critical and live-saving. While patients with suspected internal bleeding are usually monitored in hospitals, many DHF patients may be treated in clinics with minimal or no laboratory facility. Preliminary diagnosis of DHF is based on clinical features, such as fever, petechia, hepatomegaly, and hematological laboratory findings, such as high hematocrit, low platelet count, and atypical lymphocytes. [1] Increased hematocrit is a sign of hemoconcentration caused by plasma leakage that may lead to shock and death. Monitoring hematocrit is essential not only for diagnosis but also for assessing severity and determining the need for intravascular fluid in DHF patients. [1] DHF is a common mosquito-borne disease in tropical countries. Many of dengue-endemic countries are developing countries, where standard healthcare may not be easily accessible for everyone. Some rural areas may be far away from hospitals with proper basic laboratory equipment. Some healthcare providers may need to work in settings where no laboratory service is available.

Furthermore, anemia is found to be the most common disorder of the blood, which makes it even more important to diagnose it in a timely fashion. Anemia has three common causes: blood loss, red blood cells destruction, and faulty production of red blood cells from the bone marrow. All of these result in a dropped number of red blood cells. The most common causes of chronic anemia in Thailand are iron deficiency and thalassemia. Detecting anemia will help patients to get proper treatment and improve their health. Being able to measure hematocrit without using any equipment will be useful in fieldworks when a screening for anemia is needed. This present method can be used to assess the presence of anemia and facilitate further therapeutic intervention. Other disorders that can be diagnosed by measuring hematocrit include, kidney failure (low hematocrit), insufficient intake of essential nutrients (low hematocrit), burns or other serious injuries (high hematocrit) and dehydration (high hematrocrit).

Having a simple method for hematocrit estimation would be helpful for taking care of DHF patients in these settings. Evaluating patients with suspected internal hemorrhage is another example of the situations, in which hematocrit is needed in an emergency manner. In DHF patients, high hematocrit often indicates a high risk of dengue shock syndrome which, in many cases, leads to death, while lower value can indicate hemorrhage. In certain situation, such as when doctors take care of patients in a rural area, where no laboratory equipment is present, there is no ability to measure hematocrit value, therefore, the patients are at risk.

Hematocrit is usually measured by using a centrifuge to separate blood cells from plasma. Hematocrit can then be measured by indicating the percentage of erythrocyte volume in blood. Although using this normal method is easy and quick, it requires a hematocrit capillary and a centrifuge. There are various alternatives to the conventional method of using centrifuge. One is to use the measurement of blood specific gravity by floatation in copper sulfate

solution. [2, 3] This method uses copper sulfate solutions in various concentrations. Blood drops sink in solutions with lower specific gravity than blood, float on the surface of solutions with higher specific gravity than blood, and float in the middle of the solution with similar specific gravity to blood. While this method is simple, it requires accurate measurement of specific gravity of the copper sulfate solutions. The solutions need to be replaced regularly because the blood drops will mix with the solution and change the specific gravity of the solution.[2, 3] Using the idea behind the copper sulfate solution method, we can drop blood into another type of solution that does not mix with blood and let the droplet sinks to the bottom. According to the law of buoyancy, sinking objects are pulled down with a net force that is linearly correlated to the specific gravity of the objects. In viscous fluid, the gravity force is balanced by the resistance of the fluid giving a terminal velocity that is in a linear relationship with the down-pulling force. The sinking speed should therefore be linearly correlated with blood specific gravity. We tested whether this law of physics can be applied into a practical measurement of blood specific gravity.

## Materials and Methods

Anonymous blood samples were randomly gathered from leftover specimens in a hematology laboratory. These samples were gathered without any information on the donors' identification or medical conditions, and only the hematocrit values were recorded. The samples were collected in vacutainer tubes with anti-coagulant (sodium EDTA). The samples were stored at 4$^{o}$C and used within 48 hours after collection.

In order to test whether liquid droplets sink with the speeds that are determined by their specific gravity, NaCl solutions with different specific gravity were used. The solutions with specific gravity of 1.03, 1.04, and 1.05 were prepared by dissolving 3, 4, and 5 grams of NaCl, respectively, in 100 mL of distilled water. For the sinking medium, we used refined palm oil for cooking in a 1-liter bottle. The height of the oil in the bottle was 26 cm. The sinking times were measured using a digital stopwatch. Blood samples were dispensed in droplets onto the surface of the oil using a micropipette.

## Results

To start off the experiment, we decided to test whether salt solutions of various specific gravities in the range close to that of blood sink in oil with different speed in accordance to their specific gravity. Salt solutions with specific gravity of 1.03, 1.04, and 1.05 were dropped onto oil surface and the time that droplets took to reach the bottom of the bottle was recorded. The experiment was done in quadruplicate fashion. We found the time each droplet of salt solution took to reach the bottom of the tube to be in a linear relationship with its specific gravity. After the experiment using salt solutions to simulate blood showed satisfactory result, we moved on to using real blood samples, using the same experiment procedure but use real blood samples instead of salt solutions. These samples were randomly selected with the data of the blood's hematocrit values. We arranged the 23 blood samples in random order and test each samples in triplicate. Figure 1a and b shows the relationship between the time blood droplets took to reach the bottom of the bottle and their specific gravity using

the blood droplet volume of 10 and for 20 µl, respectively.

Both the experiments with 10 and 20 µl volume showed a clear linear relationship between the falling time and hematocrit with correlation coefficient of -0.86 and -0.84, respectively ($p < 0.000001$). The higher the hematocrit value, the shorter the time it took to fall. Comparing the 10 and 20 µl experiments, smaller volume had a wider falling time that ranged almost 6 seconds, making it easier and more noticeable. Measuring the falling time by a digital stopwatch was quite reproducible with a mean of standard deviations of 0.36 and 0.52 seconds for the 10 and 20 µl volume, respectively.

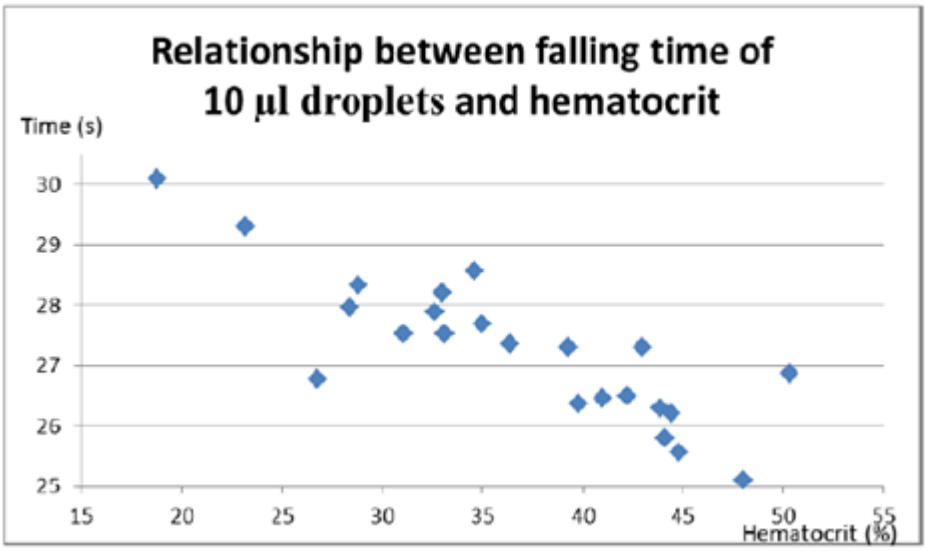


Fig 1a.

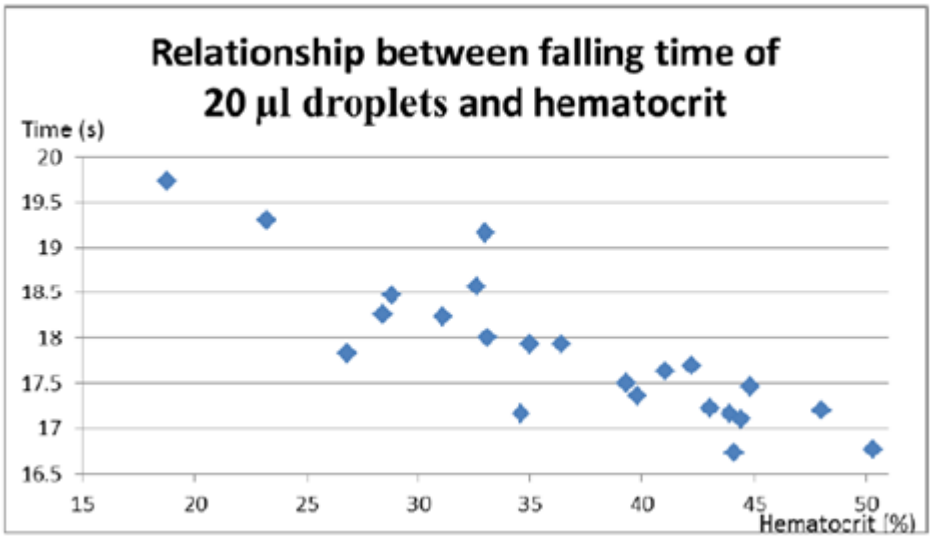


Fig 1b.

**Figure 1.** The X-axis represents the hematocrit values (%) of the blood samples, while the y-axis represents the time that droplets took to reach the bottom of the bottle (seconds). Time measurement was done in triplicate and the mean values are shown.

**Discussion**

Normally, blood consists of 54% plasma, 45% red blood cells, and less than a percent of white blood cells; these three percentages can vary, resulting in an unstable set of specific gravity. Red blood cells have a specific gravity of around 1.09, whereas plasma has a specific gravity of around 1.02.[4] Increasing hematocrit value will result in a higher fraction of the red blood cells resulting in an increase of the blood specific gravity which can be estimated by summing the mass of red blood cell (specific gravity × volume) and plasma fractions. For example, an increase in hematocrit from 45% to 50% would result in an increase of blood specific gravity from 1.051 to 1.055.

Although hematocrit directly affects blood specific gravity, there are other contributing factors, including protein concentration of plasma. Higher protein concentration in plasma also causes an increase in plasma specific gravity, hence the blood specific gravity. [5, 6] Dehydration is a common condition that increases plasma specific gravity.[7] However, DHF patients usually have some dehydration, which can worsen the reduction of blood volume. Therefore, increased plasma specific gravity is likely to be even more sensitive than increased hematocrit in detecting severe cases.

The net force exerting on the blood droplets sinking in oil is the product of weight, buoyancy force, and viscous friction. Theoretically, in a frictionless fall, the net force would equal weight (blood specific gravity × volume × g) minus buoyancy force or weight of oil with equal volume (oil specific gravity × VOLUME × g). With everything else constant, the net force in a frictionless fall would be in a linear relationship with the blood specific gravity. In reality, for a fall in viscous fluid, the down-pulling force will be negated by the viscous friction, which increases with the falling speed. At equilibrium, the terminal velocity therefore should have a linear relationship with the down-pulling force, which is in linear relationship with the specific gravity. Therefore, the falling time should be in a linear relationship with the specific gravity as we have observed in the experiments.

Our method should not be confused with erythrocyte sedimentation rate (ESR). While ESR measures the sinking rate of red blood cells in the blood, our method measures the sinking rate of blood drops in oil. ESR is used to detect systemic, chronic inflammation and other diseases, such as anemia, cancers, and autoimmune diseases. When internal inflammation occurred, the level of a protein called “fibrinogen” is significantly increased in the blood, making the red blood cells aggregate and fall down easier.

The aim of this project is to provide an alternative method for measuring hematocrit. The needed materials are basic and omnipresent in the market, except for micropipette. Nevertheless, small droplets can be easily measured and dispensed using a dropper fitted with a micropipette tip that was marked for a fixed volume. Plastic disposable micropipette tips are cheap and widely available. They can be easily marked with the preferred volume on the outside surface. The measurement requires little skill or training and should be practical. This practical measurement should be further tested in a field setting to evaluate its usefulness in patient care in resource-poor settings.

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