

The Effectiveness of Citrus Limon Extract as A Substitute for Glacial Acetic Acid Solution in The Turks Solution

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ABSTRACT

In basic clinical laboratories, the availability of chemicals to create a test solution (reagent) is frequently limited or non-existent, delaying the sample inspection procedure. In medical laboratories, the inability to create a test solution with a certain chemical compound is a common constraint. The components of Turk's reagent are glacial acetic acid, gentian violet, and distilled water. The chemical glacial acetic acid, which is required to prepare Turk's reagent for leukocyte count testing, is frequently unavailable. To confirm that the counting of leukocytes may still be performed, it is required to find a readily available replacement to glacial acetic acid solution in turk solution that serves the same purpose. This study was done to investigate the viability of substituting extract Citrus limon for glacial acetic acid in the production of Turk's solution for the measurement of leukocyte counts. The study was developed as an experiment and conducted at Prof. Dr. Hamka's Medical Laboratory Technology Laboratory at Muhammadiyah University in Jakarta. In this investigation, a total of 30 blood samples containing the anticoagulant Ethylenediaminetetraacetic acid (EDTA) were utilized. The samples were separated into three treatment groups, each containing 10 samples: lemon treatments with concentrations of 2%, 3%, and 4%. Comparing the results of the study with those of controls using Turk solution revealed that lemon juice can be substituted for glacial acetic acid in Turk solution for determining the number of leukocytes, with a concentration of 2% being optimal.

Keywords: Citrus limon; Glacial Acetic Acid ; Leucocyte; Turk Solution

ABSTRAK

Ketersediaan bahan kimia dalam laboratorium klinik sederhana untuk membuat suatu larutan uji (reagensia) sering terbatas atau bahkan tidak tersedia sehingga menyebabkan terhambat proses pemeriksaan sampel. Salah satu bahan kimia tertentu untuk membuat suatu larutan uji, merupakan

keterbatasan yang sering terjadi dalam laboratorium medik. Reagen Turk memiliki komposisi asam asetat glasial, gentian violet, dan aquadest. Salah satu bahan kimia yang sering tidak tersedia adalah asam asetat glasial yang diperlukan untuk membuat reagen Turk dalam pemeriksaan hitung jumlah leukosit. Agar pemeriksaan hitung jumlah leukosit tetap dapat dilakukan, maka perlu dicari bahan alternatif sebagai pengganti larutan asam asetat glasial pada larutan Turk serta memiliki fungsi yang sama dan mudah diperoleh. Penelitian ini dilakukan untuk mengetahui efektifitas air perasan jeruk lemon (*Citrus limon*) sebagai pengganti asam asetat glasial pada pembuatan larutan Turk dalam pemeriksaan hitung jumlah leukosit. Penelitian didesain sebagai penelitian eksperimental dan dilakukan di Laboratorium Teknologi Laboratorium Medis Universitas Muhammadiyah Prof. Dr. Hamka Jakarta. Sebanyak 30 sampel darah dengan antikoagulan Ethylenediaminetetraacetic acid (EDTA) digunakan pada penelitian ini. Sampel dibagi dalam 3 kelompok perlakuan yaitu perlakuan jeruk lemon dengan konsentrasi 2%, 3%, dan 4% dengan masing-masing terdiri dari 10 sampel. Hasil penelitian dibandingkan dengan kontrol yang menggunakan larutan Turk, hasil menunjukkan Air perasan jeruk limon dapat digunakan sebagai alternatif pengganti asam asetat glasial pada larutan Turk untuk perhitungan jumlah leukosit dengan konsentrasi terbaik air perasan jeruk limon ialah 2%.

Keywords: *Asam asetat glasial; Citrus limon; Leukosit; Larutan Turk*

INTRODUCTION

Leukocyte examination is one of the routine tests performed in a clinical laboratory. Although the use of automatic methods with haematology analyzers has been widespread in examinations and calculations of leukocyte counts, manual methods are still frequently employed in simple clinical laboratories, particularly for reference when the results obtained with automated tools are uncertain or for error checking (Chung *et al.*, 2015). It is also frequently used for single inspections because cheaper (Kementerian Kesehatan RI, 2014). Is utilized in the manual method of determining the leukocyte count.

Turk's solution, composed of glacial acetic acid solution, 1% Gentian violet solution,

and 100 mL of distilled water. In addition to leukocytes, glacial acetic acid lyses erythrocytes, platelets, and other cells. The function of gentian violet is to impart color to the nucleus and granules of leukocyte (Chandler, Mueller and Paolacci, 2014). Lime can be used to replace of glacial acetic acid in the Turk reagent for leukocyte testing (Idham, 2017). According to the research of Idham (2017), Similar to citric acid in lime, acetic acid has the ability to lyse erythrocytes and platelets due to the presence of naturally weak acid. Citrus fruit contains 7% citric acid (Rachman, 2015). Extract citrus limon contains acetic acid and limonene essential oil. Ripe citrus limon contains 8 percent acetic acid by weight (Badan POM RI, 2011).

Lemons contain acetic acid, a weak acid that is similar to citric acid, hence they have the ability to lyse erythrocytes and platelets (Gandasoebrata, 2010). Lemon juice contains 5% acetic acid, which imparts its characteristic lemon flavor, and has a pH of approximately 2-3 (Nizhar, 2012).

Lime fruit (*Citrus auratifolia* Swingle) contains a variety of compounds, including amino acids (tryptophan and lysine), citric acid essential oils (limonene, linalin acetate, geramil acetate, felandren citrate, lemon camphor, kadinen, aktialdehyd, and anildehyd), vitamin C, and vitamin B1. And lime has an acidic pH value of 2.0 (Sarwono, 2001).

In straightforward clinical laboratories, Turk reagents for counting leukocytes are frequently unavailable or have expired. To anticipate these situations, an investigation was conducted to identify alternatives to the 2% glacial acetic acid solution utilized in Turk's solution. Lemon juice with concentrations of 2, 3, and 4% is anticipated to replace glacial acetic acid with a concentration of 2%.

According to some sources, leukocytes are stable in acidic solutions up to 3 percent. If the glacial acetic acid concentration is greater than 3%, it will produce leukocyte

lysis. However, if the concentration is less than 2%, the erythrocytes and platelets will not lyse entirely (Man *et al.*, 2020). Researchers wish to determine the ideal concentration of citric acid (lemon) for lysing red blood cells as an alternative to 2% glacial acetic acid. This research was done to assess the efficacy of lemon juice as a replacement for 2% glacial acetic acid solution in Turk solution for the evaluation of leukocyte count.

METHOD

This research has received ethical approval from the ethical committee of th The UHAMKA Research Ethics Commission has issued the No. 01/19.06/046.

The materials used in this study were dry cotton, 70% alcohol cotton, tourniquet, 3cc injection syringe, serological tube, serological tube rack, Improved Neubauer counting chamber with cover glass, tissue, microscope, micropipette with 1000, 20-200, and 10 µl micropipette tips, volumetric pipette 10 mL tube and test tube rack, Erlenmeyer 250 ml, stative burette, beaker, analytical balance, knife or cutter, funnel, and Turk solution, 100 percent glacial acetic acid, 1% Gentian violet, lemon (*Citrus limon*), NaOH, 1% PP indicator, distilled water, Whatman 42 filter paper, and oxalic acid were the components employed.

East Jakarta's Klender National Housing Market was shopped for lemon samples. Purposive sampling was used to pick samples by randomly selecting lemons with rough skin, yellow color, slightly round form, and an abundance of orange liquid. Citrus Lemon (*Citrus limon*) is determined by the Center for Plant Conservation Research, Institute of Sciences, to verify that the sampled lemon is accurate. Three lemon slices were squeezed and filtered with Whatman 42 filter paper to produce Lemon Solution. In the meantime, Turk solution was made by combining 1 ml of 100% glacial acetic acid and 1 ml of 1% Gentian violet solution in a 100 ml volumetric flask, followed by adjusting the volume to 100 ml with distilled water. Carefully weighing 0.63 grams of oxalic acid with an analytical balance, it was then transferred to a 100 ml volumetric flask and dissolved with distilled water to the mark. Then 0.4 grams of NaOH was weighed and transferred to a 100 ml volumetric flask, where it was slowly dissolved with distilled water until the mark was reached. Next, 0.1 N oxalic acid was used to standardize NaOH, which was then poured into a 50 ml burette attached to a standard iron pole. Pipet ten milliliters of NaOH into an Erlenmeyer. Three drops of 1% phenolphthalein indicator were added, and the solution was titrated with oxalic acid

solution until the color of the solution changed.

In addition, the Citric Acid content of Citrus Lemon (*Citrus limon*) was determined by pipetting and adding 10 ml of distilled water to 10 ml of citrus lemon juice in an Erlenmeyer. Add two drops of pp indicator and titrate with 0.1 N sodium hydroxide until the color turns to pink. Then, the citric acid concentration is calculated using the following formula:

$$\% C_6H_8O_7 = \frac{V_x N_x f_p}{V_{Sampel}} \times 100 \%$$

After determining the levels of citric acid in lemons, proceed with diluting the juice of lemons (*Citrus limon*) calculated using the following formula:

$$V_1 \cdot \%1 = V_2 \cdot \%2$$

Information :

V1 : Stock Solution Volume

%1 : Stock Solution Concentration

V2 : Volume of Treatment Solution

%2...:Desired Solution Concentration

To make 2% extract Citrus limon is done by pipetting 3.5 ml of extract Citrus limon and put it in a 10 ml volumetric flask and add it to the limit with distilled water. Meanwhile, to make 3 and 4% lemon juice (*Citrus*

limon), then pipette 5.3 and 7 ml of lemon juice (Citrus limon) respectively.

Next, prepare a Turk solution of 2, 3, and 4% Lemon Juice (Citrus limon) by dissolving 1 ml of each concentration of 2, 3, and 4% Lemon Juice (Citrus limon) in 1 milliliter of Gentian violets 1%. Then, 100 cc of distilled water were added to the mixture. Gentian violet 1% is used to color the granules in leukocytes so they can be counted more easily. In order to obtain samples from the respondent's vein, the arm was first immobilized using a tourniquet. In a circular motion, 70% alcohol disinfectant cloth is applied to the target area. Up to 3 ml of venous blood is aspirated and then slowly poured through the tube wall into an Ethylenediaminetetraacetic acid (EDTA) tube. Blood is homogenized by shaking the tube into the shape of an eight. Next, pipette 190 l of Turk solution containing 2, 3, and 4% lemon juice (Citrus limon) into a serological test tube. Transfer 10 ul of blood to a Turk's solution-filled serological tube using a micropipette. The leftover blood in the micropipette tip was rinsed three times with Turk's solution and homogenized by sucking and removing the solution for 15 to 30 seconds.

The counting chamber is prepared by moistening the borders with water so that the

cover slip can be securely adhered. The counting chamber is protected by a glass cover. The solution is sucked out using a micropipette, then the tip of the micropipette is inserted in the counting chamber at an angle of 30 degrees, touching the edge of the cover glass, and the counting chamber is filled with liquid by capillary action. The counting chamber is left for two to three minutes to allow the leukocytes to precipitate before being counted. Leukocytes were counted utilizing a counting chamber that was horizontally positioned on a microscope preparation table. Leukocyte cell count was examined using a 10x and 40x lens, with the condenser lowered and the iris diaphragm closed. Leukocytes were counted in the four huge fields of leukocytes at the chamber's four corners. Beginning in the upper left corner, continue horizontally to the right, then descend, continue horizontally to the left, then descend and continue horizontally to the right, etc. This procedure is performed on four huge fields of leukocytes. However, cells that contact the right and bottom lines are not tallied. The true value is derived by adding the cell counts from the four major chambers and multiplying by 50. (Gandasoebrata, 2010).

RESULTS AND DISCUSSION

Assembly of Lemon Orange Solution (Citrus limon)

The result of squeezing three lemons (Citrus limon) is 100-150 ml of lemon juice, which is expressed as a viscous lemon juice that will be employed as the mother liquor. In addition, from this mother liquor, an analysis was conducted to evaluate the citric acid content of lemons (Citrus limon).

Evaluation of Citric Acid Concentrations in Citrus Lemons (Citrus limon)

The citric acid concentration of Citrus limon was determined using the acid-alkalimetric titration technique (Andari, 2013). The findings of measuring the citric acid content of lemons (Citrus limon) are displayed in Table 1.

Table 1. Results of Determination of Lemon Citric Acid Levels (Citrus limon)

No.	Volume of Citrus limon extract (ml)	Volume of NaOH (ml)	Citric Acid Rate (%)
1	10	8,5 ml	5,6412
2	10	8,6 ml	5,7076
3	10	8,8 ml	5,8403
Mean of Citric Acid Rate			5,7

Table 1 shows the results of the average citric acid content found in lemons (Citrus limon) is 5.7%. which can later be diluted to be used as a substitute for 2% glacial acetic acid in Turk's solution. The citric acid levels obtained are in accordance with the literature that the levels of citric acid in lemons (Citrus limon) are 5% (Nizhar, 2012). The result that displayed in table 1 of squeezing three lemons are 100-150 ml of lemon juice, which is expressed as a viscous

lemon juice that will be employed as the mother liquor. In addition, from this mother liquor, an analysis was conducted to evaluate the citric acid content of lemons (Citrus limon). Table 1 reveals that lemons (Citrus limon) contain an average of 5.7% citric acid, which can be diluted and used as a substitute for 2% glacial acetic acid in Turk's solution. According to the scientific literature, the citric acid levels in lemons (Citrus limon) are 5%. The citric acid levels

that we measured correspond to this value (Nizhar, 2012)

Standardized Citrus Limon Dilutions



Figure 1. Results of Making Turk Solution 2, 3, and 4% Citrus Lemon extract

Table 2: Standardized Citrus Limon Dilution Outcomes

Citrus Lemon Concentration	Acid Pipetting (ml)	Preliminary Liquid (ml)	Volume
2%	3,5 ml	10 ml	
3%	5,3 ml	10 ml	
4%	7,0 ml	10 ml	

According to table 2, lemons (Citrus limon) with known quantities or levels are diluted to 2, 3, and 4%, respectively. A 2% dilution of each lemon juice solution (Citrus limon) is taken at 3.5 ml and diluted with 6.5 ml of distilled water, or up to a maximum of 10 ml of distilled water. Then, for the 3% dilution, 5.3 ml was collected, and for the 4% dilution, 7.0 ml was obtained, to which 10 ml of distilled water was added.

The concentrations of 2, 3, and 4% lemon juice (Citrus limon) chosen correspond to

the concentration of glacial acetic acid used in Turk's solution to count leukocytes, which is 2%. If the acid level is less than 2%, blood cells do not precipitate or lyse; if it is greater than 3%, all proteins or blood cells, including leukocytes, will be lysed (Man *et al.*, 2020). Based on this statement, lemon juice (Citrus limon) is diluted between 2-3% and 4% as a control to see whether all blood cells are lysed at a concentration of 4%.

In the treatment, 1 ml of lemon juice solution (Citrus limon) was combined with

1 ml of 1% Gentian violet solution, followed by the addition of 100 ml of distilled water. Leukocytes that have not been lysed are stained with Gentian violet 1%, which turns the granules of the leukocytes blue so that they may be easily counted in an Improved Neubauer counting chamber. If leukocytes are not stained, leukocytes cannot be counted (Afrianti, 2016). The results of making a Turk solution of 2, 3, and 4% lemon juice (Citrus limon) can be seen in Figure 2.

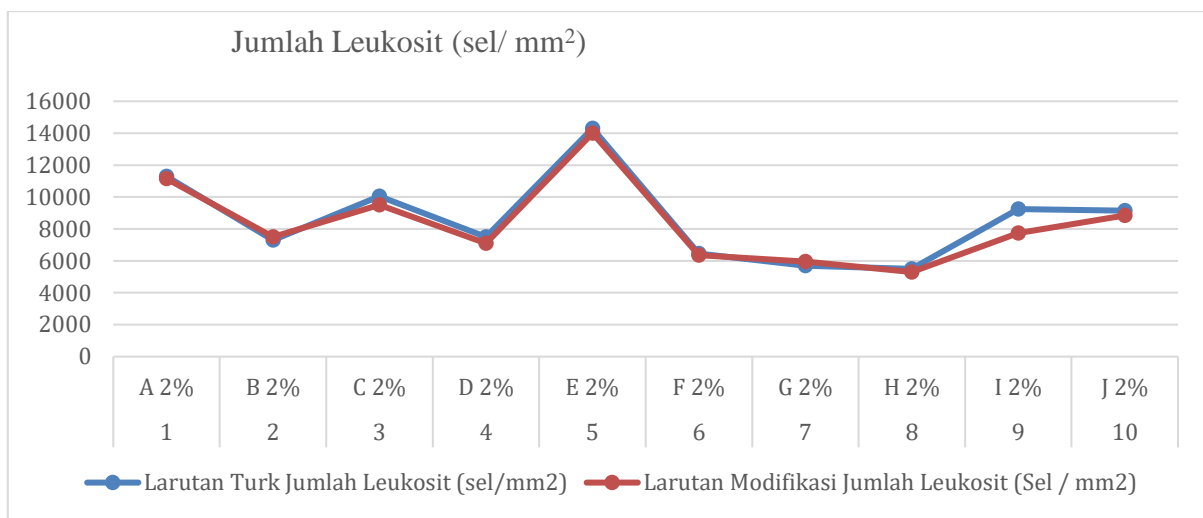
Collection of Venous Blood

The blood sample utilized was anticoagulant-treated venous blood. EDTA prevents blood clot formation. Blood samples taken as many as 30 samples. This blood sample will be utilized for the test treatment with as many as 10 samples of 2% modified Turk of lemon juice (Citrus limon). 10 blood samples will be treated with 3 and 4% lemon juice (Citrus limon) for the subsequent sample.

Test Sample Measurement

The test sample of lemon juice (Citrus limon) was created by changing the current Turk solution by substituting 2,3 and 4% lemon juice (Citrus limon) for the glacial acetic acid used in the Turk solution. The treatment was initiated by pipetting 190 l of a 2% lemon juice (Citrus limon) solution into 10 l of blood and then homogenizing the mixture. The solution is then sucked into the counting chamber, which is covered with glass, at a 30° angle using a micropipette. The solution will fill the counting chamber to the point where it contacts the glass. 15 to 30 seconds are allowed for the leukocytes to settle in the solution. If the sample has not yet been inspected, the counting chamber containing the sample is placed in a petri dish with moist tissue or filter paper to prevent it from drying out. Examining the quantity of leukocytes involved counting leukocytes in four huge rooms within the counting room. There are cells that are positioned outside of a field's perimeter. The cells that violate the left or top boundary lines must be tallied. Cells touching the right or bottom border should not be counted (Gandasoebrata, 2010).

Calculation of the Number of Leukocytes with Turk and Turk Solution 2% Citrus Lemon Extract



Graphic 1. Leukocyte Count Calculation Results with 2% Citrus Limon Extract Turk

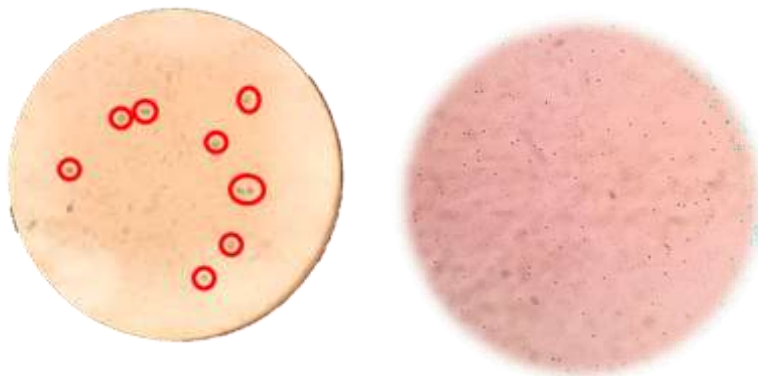


Figure 2: Microscopic Results of Turk Solution on Leukocytes 2% Citrus Limon Extract Turk and Control

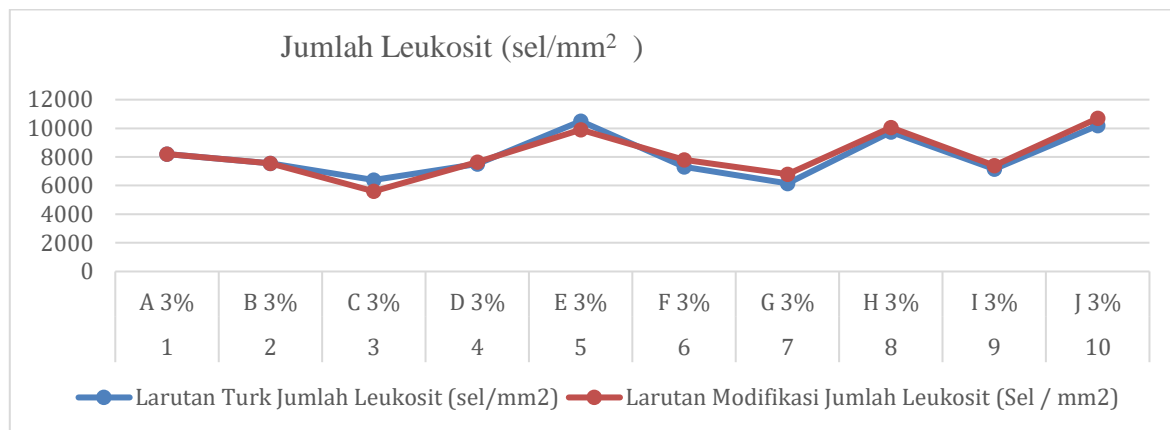
The results of the number of leukocytes obtained using a 2% lemon juice (Citrus limon) Turk solution can be seen in Graphic 1. From Graphic 1, it can be seen that the

graph between Turk standard solution and 2% Citrus limon juice has almost the same pattern. This suggests that a 2% solution of Turk lemon juice (Citrus limon) has the

potential to be used as a substitute for 2% glacial acetic acid in Turk solution to calculate the number of leukocytes. The results show that leukocyte cells are still stable at levels of 2% so that leukocyte cells are still visible and easy to count (Man *et al.*, 2020). Figure 2 demonstrates that neither Turk solution in 2% citrus limon juice resulted in the presence of non-leukocyte cells when observed microscopically. There is a risk of contamination in the outcomes of this study, however this possibility is tiny. Due to the smaller amount of lemon (Citrus limon) used, contamination of other cells is

minimal. This demonstrates that with 2% lemon juice (Citrus limon) Turk solution, erythrocytes and platelets lyse, leaving only leukocytes visible; hence, 2% lemon juice (Citrus limon) Turk solution can be used as a substitute for 2% glacial acetic acid in Turk solution. to count the leukocyte count. In the study, (Castaño *et al.*, 2022) also employed Citrus Limon juice as a replacement for glacial acetic acid in Turk solution, although the concentration of the Citrus Limon solution was not specified (Castaño *et al.*, 2022).

Calculation of the Number of Leukocytes with Turk and Turk Solution 3% Citrus Lemon Extract



Graphic 2. Leukocyte Count Calculation Results with 3% Citrus Limon Extract Turk

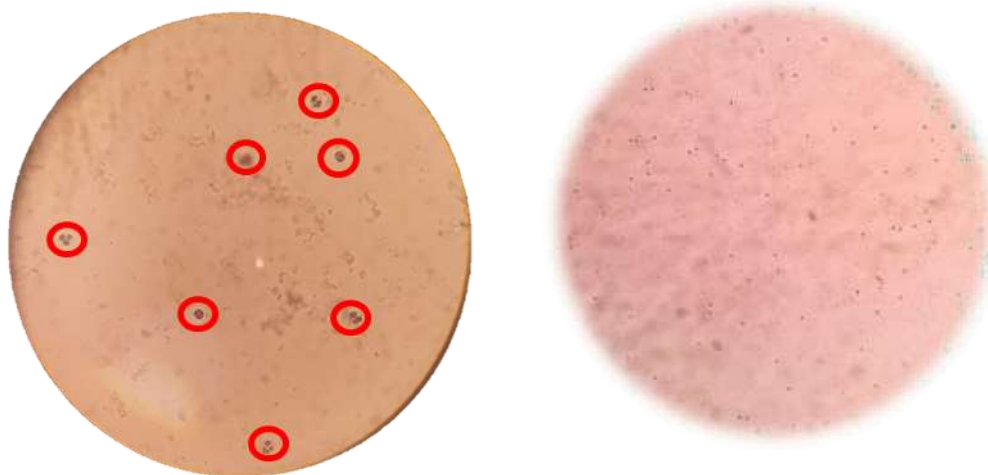
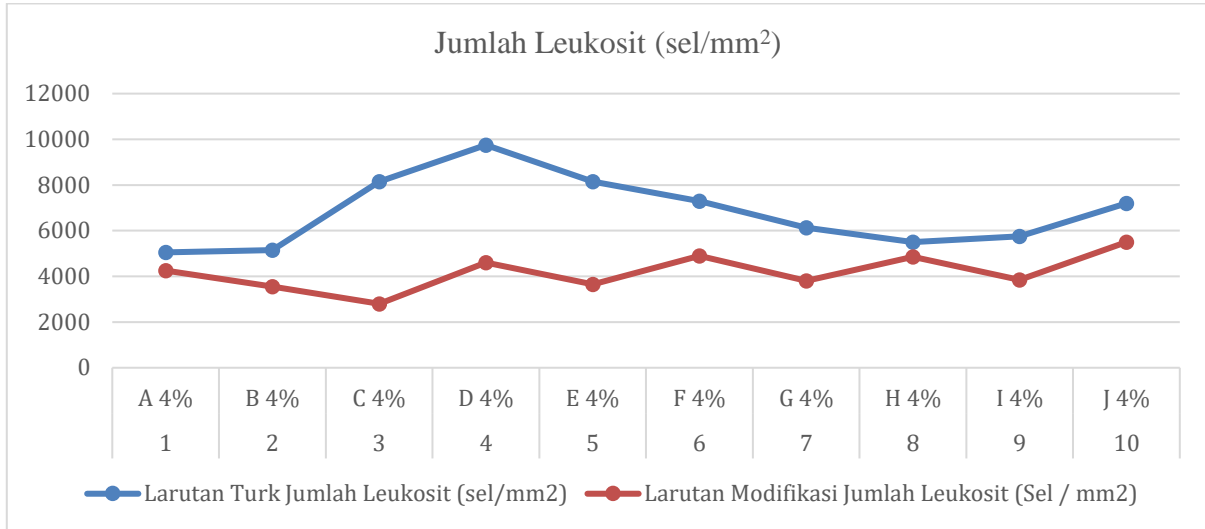


Figure 3: Microscopic Results of Turk Solution on Leukocytes 3% Citrus Limon Extract Turk and Control

Graphic 2 reveals that the graphs for Turk standard solution and 3% lemon juice (Citrus limon) Turk solution have almost identical patterns and are nearly identical. This indicates that a 3% solution of Turkish lemon juice (Citrus limon) may be used for 2% glacial acetic acid in Turkish solution for calculating the number of leukocytes. According to the scientific literature, leukocytes are still stable up to a concentration of 3% and cause erythrocyte and leukocyte lysis (Man *et al.*, 2020). The data obtained indicate that leukocytes are still detectable under the microscope. Due to

the greater volume of the diluted 3% lemon (Citrus limon) solution compared to the 2% lemon (Citrus limon) solution, other contaminants, such as dirt fibers from the lemon, remain visible. This demonstrates that a 3% solution of Turkish lemon juice (Citrus limon) causes the lysis of erythrocytes and platelets. However, lemon juice (Citrus limon) still contains fiber, hence a 2% lemon juice (Citrus limon) Turk solution is recommended for counting leukocytes. This is done to prevent inaccuracies in leukocyte count calculations (Khan *et al.*, 2012).

Calculation of the Number of Leukocytes with Turk and Turk Solution 4% Citrus Lemon Extract



Graphic 3. Leukocyte Count Calculation Results with 3% Citrus Limon Extract Turk

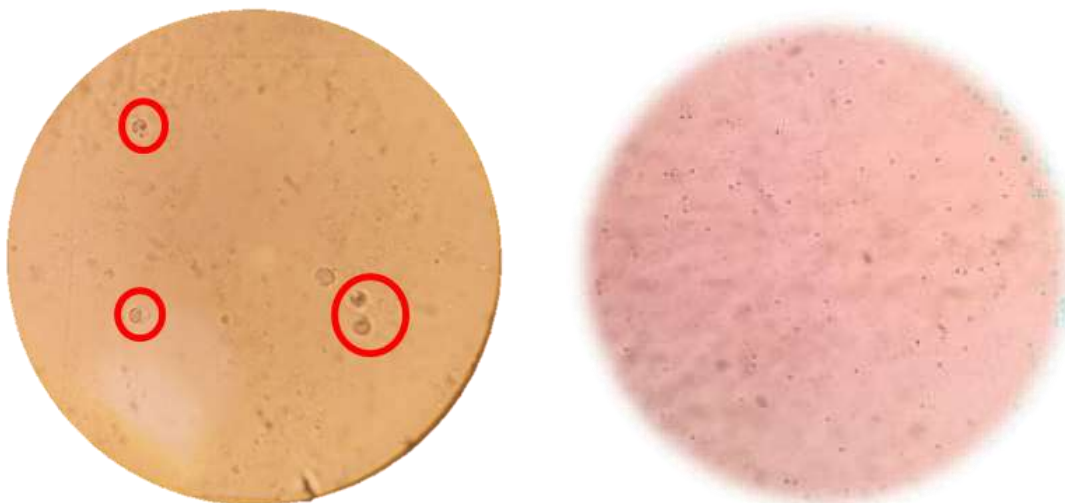


Figure 4: Microscopic Results of Turk Solution on Leukocytes 4% Citrus Limon Extract Turk

Graphic 3, shows that the graph between Turk's conventional solution and his 4% lemon juice (Citrus limon) solution has a significantly different trend. This indicates that a 4% solution of Turkish lemon juice (Citrus limon) cannot replace 2% glacial acetic acid in a normal Turkish solution for

counting leukocyte counts. Literature demonstrates that leukocytes lyse at acid concentrations exceeding 3%. (Man *et al.*, 2020).

Figure 4 demonstrates that the amount of leukocytes in ordinary Turk solution diluent

and Turk lemon juice (Citrus limon) 4% are distinct. It can be observed that the Turk solution of 4% lemon juice (Citrus limon) contains less leukocytes than the standard. This is due to the fact that 4% lemon juice (Citrus limon) Turk solution lyses not only erythrocytes and platelets, but also certain leukocytes. Turk solution of 4% lemon juice (Citrus limon) cannot be substituted for 2% glacial acetic acid while counting the quantity of leukocytes. Other cell contamination is still obvious because the volume diluted with lemon juice is greater than 2% or 3%. (Citrus limon)

CONCLUSION

Extract Citrus limon 2% may be used for glacial acetic acid in Turk solution for counting leukocytes. For next, researchers can modify citrus limon extract with a concentration of less than 2%.

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