COMPARISON OF LEUKOCYTE COUNTING METHODS OF HEMATOLOGIANALYZER AND HEMOCYTOMETER IN TOTO HOSPITAL KABILA BONEBOLANGO

Junelan Ningsih AS Pandju[1)], Rita Amini Warastuti[2)], and Srikit Nurkamiden[3)]
[1,2,3)] Bina Mandiri University of Gorontalo
E-mail:nelanpandju22@gmail.com

ABSTRACT

Examination of leukocyte count is a routine blood test that is widely requested in health care units, both clinics, health centers or even in hospitals. This study aims to establish the diagnosis of the disease. One laboratory examination, namely a hematological examination, to find out the comparison of leukocyte counts with the Hematology Analyzer and Hemocytometer methods at Toto Kabila Hospital, Bone Bolango Regency.

The type of research used is Observational Analytic Research with a quantitative approach, analytical research is research that looks for relationships between variables, namely by analyzing comparisons. The sample size taken was 30 samples with the sampling technique using purposive sampling. This type of research wanted to see the results of a comparison of leukocyte counts using the hematology analyzer and hemocytometer methods at Toto Kabila Hospital, Bone Bolango Regency.

The results obtained during this research areThe results of a comparison of leukocyte examination using a hematology analyzer and hemocytometer were obtained0.039 <0.05, where the null hypothesis (H0) means rejected and the alternative hypothesis (Ha) means accepted, so it can be concluded that the data obtained is different.

Keywords: Leukocytes, Hematology Analyzer, Hemocytometer

INTRODUCTION

Laboratory Examination is an examination carried out for clinical purposes. The purpose of laboratory examination is to establish a disease diagnosis [6].

One of the laboratory tests is a hematological examination. Hematology is the study of examining the condition of peripheral blood cells under normal and pathological conditions [6].

One of the parameters of the hematological examination is the complete blood count which consists of examination of the sedimentation rate, hemoglobin, hematocrit, count of erythrocytes, count the number and types of platelets, count the types of leukocytes [6].

Examination of leukocyte count is a routine blood examination that is widely requested in health care units, be it clinics, health centers or hospitals, this is due to the increasing need for these tests which aim to provide information about various disease states in an effort to help make a diagnosis [11].].

There are two methods for checking the leukocyte count, namely the manual method and the electronic or automatic method. Many electronic or automatic methods have been carried out using a blood cell counting machine (hematology analyzer) [10].

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The basic principle used, namely impedance (Electrical Resistance). The principle of impedance is based on detecting and measuring changes in the electrical resistance produced by blood cells as they pass through a small hole (aperture). The leukocyte count with the analyzer will be displayed on the result sheet as Whole Blood Cell (WBC) [10].
The use of the Automatic method with a blood cell counter is more beneficial because it is able to count cells in much larger numbers, saves time and effort and fast results can be accepted by clinicians for the benefit of therapy in patients [10].

In large laboratories with large workloads, these efforts are carried out by using automatic calculating tools which give results that are easier, faster, and more accurate than the manual method, however the use of automatic tools is only limited to certain laboratories or large clinics with The reason is that the Hematology Analyzer tool is quite expensive and requires very careful use and maintenance, besides that it is necessary to ensure that the tool works precisely in a quality assurance program (Quality Control) [7].
With the increasing availability of automatic counters, manual method calculations are increasingly rarely carried out in the laboratory, however, the manual dilution method and visual examination of the hemocytometer are still reliable.

As long as it's done carefully. The manual method is usually carried out to inform the results of electronic or automatic white blood cell counts that are too low or too high [11].

The manual method of leukocyte examination consists of two methods, namely the Thoma pipette and the tube method. The principle of examining the tube is the same as using a Thoma pipette and leukocytes are counted visually using an Improved Neubauer counting chamber and examined under a microscope with 10x magnification [10].

The principle of the manual method is that the blood is diluted with a diluent solution, apart from the leukocytes being lysed and the blood becomes dilute so that the leukocytes are easy to count. Leukocytes per microliter of blood are counted under a microscope and then multiplied by a certain multiplier [10].

With different calculation methods (manual method and automatic method), it turns out that each has advantages and disadvantages. Therefore, it is necessary to know the results generated by the two methods, each of which has limitations [1].

From the description of the problems mentioned above, the authors feel interested in conducting research on the comparison of the results of the manual leukocyte count examination using a counting chamber (Improved Neubauer) and the automatic Hematology Analyzer method which will be carried out at Toto Kabila Hospital, Bone Bolango Regency [1].

Blood is a liquid tissue that contains two parts, namely blood cells and blood plasma. Blood cells are divided into three types namely erythrocytes, leukocytes, and platelets. The total blood volume is 1/12 body weight or approximately 5 liters. Approximately 55% consists of blood plasma, and 45% is blood cells [4].

Blood has 2 components consisting of blood particles and blood plasma. Blood plasma is the liquid part of blood consisting mostly of blood protein, water, electrolytes. Blood corpuscles have 3 elements, namely leukocytes (white blood cells), platelets (clots), and erythrocytes (red blood cells) [4].

Blood is a tissue in the body that has different properties from other tissues, it is liquid and red in color. And has many functions, including flowing oxygen throughout the body. Blood has several components, including red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (platelets) [4].

Blood plasma is the watery part of blood without blood cells, the color is clear, yellowish, and nearly 90% of blood plasma consists of water. Substances contained in blood plasma, namely, fibrinogen which functions in the blood clotting process, mineral salts (calcium salts, potassium, sodium and others) which are useful as metabolism and also osmotic, blood proteins (albumin, globulin) can increase blood viscosity and also cause osmotic pressure to maintain fluid balance in the body, nutrients (amino acids, glucose, fat, minerals and vitamins), hormones, is a substance produced by the body's glands, plasma antibodies are obtained by rotating blood cells, Plasma is given intravenously to restore blood volume, providing substances lost from the client's blood.

Erythrocytes are biconcave cells with a diameter of about 7 microns. The biconcavity allows oxygen to enter and leave the cell quickly with a short distance between the membrane and the cell nucleus. Reddish yellow in color, because it contains a substance called hemoglobin [1].

Erythrocytes do not have a cell nucleus, mitochondria and ribosomes, and cannot move. These cells are unable to carry out, cell oxidative phosphorylation, and protein formation. Erythrocyte parts, namely the erythrocyte membrane, the enzyme G6PD (Glucose6-Phosphatedehydrogenase) and Hemoglobin [1].

Platelets are blood cells that have an important role in hemostasis, platelets attach to the endothelial lining of torn blood vessels (wounds) by forming a platelet plug. Platelets do not have a cell nucleus, have a size of 1-4 microns, and their cytoplasm is blue with reddish purple granules [1].

Platelets are derived from megakaryocytes, which originate from fragments of the megakaryocyte cytoplasm. The number of platelets in the blood is 150,000-350,000/ml of blood. Platelet granules contain blood clotting factors, adenosine diphosphate (ADP) and adenosine triphosphate (ATP), calcium, serotonin, and catecholamines. Most of them have a role in stimulating the start of the blood clotting process. While the age of platelets is around 30 days [1].

Leukocytes or white blood cells have different cell characteristics, in general leukocytes are larger in size than erythrocytes, are colorless and can move in the presence of pseudo legs with a life span of 13-20 days. The number of leukocytes is the least of the three types of blood cells in the body, around 4,000-11,000/mm3. There are five types of leukocytes, namely neutrophils, eosinophils, basophils, monocytes and lymphocytes [9].

As a means of defense of the body, white blood cells function to help the body fight various infectious diseases. White blood cells consist of granulocytes and agranulocytes. Granulocytes consist of neutrophils, eosinophils and basophils. Neutrophils function against bacteria and fungi, eosinophils fight larger parasites and modulate the inflammatory response with allergies, and basophils release histamine to induce an inflammatory response [5].

This type of agranulocyte consists of lymphocytes and monocytes. There are three types of lymphocytes: B cells, T cells and natural killer (NK) cells. B cells release antibodies. Meanwhile, T cells function to bring the body back to normal after receiving an inflammatory response, they can activate and regulate B and T cells, or they can attack virus-infected cells [5].

Natural killer cells attack virus-infected cells. Monocytes migrate to tissues and then differentiate into macrophages. Macrophages are phagocytic cells, which feed on cellular waste and pathogens.

Macrophages also function to stimulate lymphocytes [5].

Neutrophils are granular leukocytes whose core has many lobes, so they are called polymorphonuclear. Constitutes 60-70% of the total number of leukocytes. These leukocytes are quite large, which is 2x the size of erythrocytes, and are able to move actively in the blood vessels and outside the blood vessels [2].

Eosinophils are more responsive to inflammation and injury than other leukocytes and are the front line of defense during the acute phase of infection. Segments are immature neutrophils that multiply rapidly during acute infection [2].

Eosinophils, namely granular leukocytes, have 2 lobes in their core, constituting 1-2% of the total number of leukocytes. These leukocytes will increase in number in the blood in the event of allergies and infections (especially worms) in the body. By administering steroids the number of eosinophils will decrease [2].

Basophils are leukocytes whose core contains large granules having the letter S, constituting 0.5-1% of the total number of leukocytes. Basophils are present in inflammatory processes, leukemia, and the healing phase of infection [2].

Lymphocytes Non-granular leukocytes with large nuclei, slightly larger in size than erythrocytes, produced by lymphatic tissue, play an important role in the immune process and the formation of antibodies [2].

Monocytes are leukocytes with non-granular cytoplasm, large nuclei with a size twice the size of erythrocytes, are the largest in the blood circulation, and are made in the lymphatic tissue [2].

A hematology analyzer is a tool used to examine complete blood by automatically counting and measuring blood cells based on variations in the impedance of the electric current (light beam) against the cells that are passed [3].

This tool works on the principle of flow cytometer. Flow cytometer is a method of measuring the number and properties of cells covered by the flow of liquid through a narrow slit. Thousands of cells pass through the gap in such a way that cells can be passed one by one, then the number of cells and their size are calculated [3].

The working principle of impedance is based on detecting and measuring changes in the electrical resistance generated by blood cells when they pass through a small hole (operture). the cell itself. The results of the leukocyte count with the analyzer are displayed on the result sheet as WBC [3].

Hemocytometer is a tool used to count the number of blood cells and which consists of a counting chamber, cover glass and two kinds of pipettes, and. The quality of the counting chamber and micro pipette must meet certain accuracy requirements [3].

In large laboratories with large workloads, these efforts are usually carried out using electronic counters. Basically such tools which are usually used with automatic diluents give very precise and precise results. Often an electronic counter is associated with a small computer that can provide data on the average leukocyte volume [3].

Blood is diluted with Turk's solution, blood cells other than leukocytes will be destroyed by acetic acid and leukocytes will be stained with gentian violet. The number of leukocytes in the dilution volume is counted using a counting chamber [8].

It was found that the number of patients undergoing leukocyte examination increased every year, reaching 10.5-24.6% of all patients. An additional 2.1-7.4 million people are admitted annually for critical illness, representing an increase of 100,000 people [12].

RESEARCH METHODOLOGY

In completing this research, 2 research locations were taken, namely inHOSPITAL. Toto Kabila as a place for sampling and examination of samples and the Bina Mandiri University Gorontalo Laboratory for sample examination.Research processis descriptive observational analytic which aims to collect data in the form of leukocyte levels by measuring on 2 different tools, namely Hematology Analyzer and Hemocytometer.

As for techniquesampling is done with*Purposive Sampling*.The research process takes placefor 21 days from July 14 to August 5 2022. Before getting the results of leukocyte levels in patients who carried out the examination, the researchers met directly with the patients to provide an explanation regarding the aims and objectives of this study and were asked for the patient's willingness to participate as respondents in this study.

If the pregnant woman agrees, then informed consent is given which is then filled out and signed by the respondent. After the patient filled out and signed the informed consent and questionnaire, the researcher checked the completeness of the contents of the questionnaire and the informed consent, then the researcher took venous blood from the respondents.The number of samples or respondents obtained was 30 people shown in table 1 above.

The venous blood that was taken was then put by the blood sample researcher into the K3EDTA tube and mixed (mixed) so that the blood is mixed homogeneously and perfectly with the anticoagulant in the tube.

Then measure leukocyte levels using a branded Hematology AnalyzerDirui BCC-3600 and Hemocytometer,The results of the leukocyte levels obtained by the researcher were then recorded on the result sheet that the researcher had prepared.

RESEARCH FINDINGS

Univariate analysis

Table 1. Frequency Distribution of Leukocyte Examination Using a Hematology Analyzer

Leukocyte Results with a Hematology Analyzer	Frequency (n)	Percentage (%)
Normal	13	43,3
Abnormal	17	56,7
Total	30	100

Source: Primary Data, 2022

Based on the results of table 1 above, it shows that out of 30 respondents, leukocyte examination using a hematology analyzer consisted of 13 normal results and 17 with results.

Table 2. Frequency distribution of leukocyte examination using a hemocytometer

Leukocyte Results with Hemocytometer	Frequency (n)	Percentage (%)
Normal	21	70
Abnormal	9	30
Total	30	100

Source: Primary Data, 2022

Based on the results of table 2 above, it shows that out of 30 respondents, leukocyte examination using a hemocytometer consisted of 21 normal results and 9 with abnormal results.

Table 3. Distribution of the frequency of leukocyte examination results using a Hematology Analyzer and Hemocytometer in RSUD. Toto Kabila

Check up result	Hematology Analyzer		Hemocytometer		Means
	F	%	F	%	12,227
Normal	13	43,%	21	70, %	
Abnormal	17	56,%	9	30, %	11.40

Amount	30	100 %	30	100 %

Source: Research Primary Data, 2022

Based on table 3 it is known that the leukocyte examination using a hematology analyzer has an average value of 12,227/mm3, with a normal value of 13 and an abnormal level of 17 with a frequency of 30 samples which has a percentage of 100%, while an examination using a hemocytometer has an average of 11,140/mm3, with a normal value of 21 and an abnormal of 9, with a frequency of 30 samples which has a percentage of 100%.

Bivariate Analysis

Table 4. Normality test results on leukocyte examination usinghematology analyzer and hemocytometer

	Shapiro-Wilk		
Leukocyte Levels Results	***Statistics***	**Df**	**Sig**
Hematology Analyzer	.632	30	.000
Hemocytometer	.577	30	.000

Source: Research Primary Data, 2022

Based on table 4 the results of the normality test, namely .000 <0.05. So it can be concluded that the data obtained is not normally distributed so that it can be continued in the Non-Parametric comparative analysis test, namely the Mann-Whitney Test.

Table 5. Mann-Withnay Test Results on Leukocyte Examination using a Hematology Analyzer and Hemocytometer

Examination of Leukocytes with a Hematology Analyzer and Hemocytometer	Significant (2-Tailed)	Significant Level	Information
	0.039	0.05	Significant

Source: Research Primary Data, 2022

Based on table 5 above, it shows that the results of the comparative analysis on the comparison of leukocyte levels using a Hematology Analyzer and Hemocytometer are 0.039 <0.05, from the results obtained the null hypothesis (H0) means rejected and the alternative hypothesis (Ha) means accepted, so it can be concluded that the data obtained there are differences.

DISCUSSIONS

In completing this research, 2 research locations were taken, namely inHOSPITAL. Toto Kabila as a place for sampling and examination of samples and the Bina Mandiri University Gorontalo Laboratory for sample examination.

Examination of leukocytes in the hospital. Toto Kabila is included in routine blood tests. Routine blood tests using a hematology analyzer of 2 types, namely Dirui and Nihon Kohden. The hematology analyzer is used alternately every week. Routine blood tests are carried out for inpatients and outpatients according to the request form provided by the doctor.

Research processis descriptive observational analytic which aims to collect data in the form of leukocyte levels by measuring on 2 different tools, namely Hematology Analyzer and Hemocytometer. As for techniquesampling is done with*Purposive Sampling*.The research process takes placefor 21 days from July 14 to August 5, 2022.

Before getting the results of leukocyte levels in patients who carried out the examination, the researcher met directly with the patient to provide an explanation of the aims and objectives of this study and asked the patient's willingness to participate as a respondent in this study.

If the pregnant woman agrees, then informed consent is given which is then filled out and signed by the respondent. After the patient filled out and signed the

informed consent and questionnaire, the researcher checked the completeness of the contents of the questionnaire and the informed consent, then the researcher took venous blood from the respondents.The number of samples or respondents obtained was 30 people shown in table 1 above.

The venous blood that was taken was then put by the blood sample researcher into the K3EDTA tube and mixed (mixed) so that the blood was mixed homogeneously and perfectly with the anticoagulant in the tube, then measured leukocyte levels using a Hematology Analyzer with the brand nameDirui BCC-3600 and Hemocytometer,The results of the leukocyte levels obtained by the researcher were then recorded on the result sheet that the researcher had prepared.

Leukocyte Levels on the Hematology Analyzer

In detecting leukocyte levels in the human body, usually in health institutions, such as clinical laboratories and hospitals, a hematology analyzer is used. In this study, researchers used a hematology analyzer brand Dirui Turkey Type BCC-3600.

The principle of measuring Hemoglobin (Hb) levels using a hematology analyzer, namely heme (ferrous) is oxidized by potassium ferricyanide to (ferric) methemoglobin, then methemoglobin reacts with cyanide ions to form cyanmethemoglobin which is brown in color. The absorbance is measured with a colorimeter or spectrophotometer at λ 540 nm.

In this method, hemoglobin is oxidized by potassium ferrocyanide to form methemoglobin which then reacts with cyanide ions to form cyanmethemoglobin which is red in color. Color intensity is read with a photometer and compared with a standard. Because those who compare electronic devices, the results are more objective [8].

Based on the research results obtained, table 2 above shows that out of 30 respondents, leukocyte examination using a hematology analyzer consisted of 13 normal results and 17 abnormal results. It can be seen that patients who have leukocyte levels checked at the RSUD. Toto Kabila mostly has leukocyte levels above the normal value, namely > 4,500 – 10,000 cells/mm3.

The choice of the automatic method or the Hematology Analyzer tool in this study is due to the fact that this automatic method is the best method. Examination of leukocyte levels with a hematology analyzer is easy to do and the examination results are more accurate than other methods.

Leukocyte Levels on Hemocytometer

Similar to the examination of leukocytes on a hematology analyzer, the researchers also examined leukocytes using a hemocytometer. Based on the results of table 4.3 above, it shows that out of 30 respondents, leukocyte examination using a hemocytometer consisted of 21 normal results and 9 abnormal.

Examination of the leukocyte count using the manual improved neubauer method at the Kendari City General Hospital as many as 25 people (83.4%) patients had a normal leukocyte count and as many as 5 people (16.6%) patients had an abnormal leukocyte count.

The hemocytometer tool used by researchers is a tool used to countblood cell count and which consists of a counting chamber, cover slip and two kinds of pipettes.

The working principle of this hemocytometer is that the blood is diluted with Turk's solution, the blood cells are separatedleukocytes will be destroyed by acetic acid and leukocytes will be stained by gentian violet. The number of leukocytes in the dilution volume is counted using a counting chamber [9].

Until now, the hemocytometer is still often used at the health facility level, such as the puskesmas. This is because the hemocytometer has advantagescan count the number of cells thatlifenor thosedead,morphologycells can be observed, can evaluatehomogeneityand detect datacontamination [1].

Based on the results of the research listed in table 5 above, it shows that from the results of a comparative analysis on the comparison of leukocyte levels using a Hematology Analyzer and Hemocytometer, it is 0.039 <0.05, from the results obtained the null hypothesis (H0) means rejected and the alternative hypothesis (Ha) which means accepted, so it can be concluded that the data obtained is different.

Examination of the leukocyte count using the manual improved Neubauer method and the automatic hematology analyzer method showed that there was a significant difference between the two methods. Testing the data using the parried sample test obtained results, p = 0.000, so it can be concluded that Ho was accepted and Ha was rejected, so there was a difference in the results of the leukocyte count manually improved Neubauer with an automatic hematology analyzer.

There are differences between the hematology analyzer and hemocytometer due to the advantages and disadvantages of each tool. In counting leukocyte cells in the improved Neubauer manual tool it is very difficult to control or get accuracy and precision, because leukocyte cells mix with dirt on glass objects, so when reading the count the number of leukocytes takes quite a long time to obtain maximum results.

Another error when using the hemocytometer is that during the analytical process, namely from the wrong dilution in the pipette, this is caused by the liquid not being sucked right at line 11, the loss of liquid from the pipette, which is due to flowing back into the bottle containing the turk solution, there is air bubbles in the pipette when the turk solution is sucked.

for the amount or volume of blood that is sucked into the pipette it is not correct, when reading on the microscope there is dirt in the counting chamber and the cover glass due to not being cleaned properly before use, wrong counting of cells that touch the boundary lines, and visible air bubbles entering along with liquid.

In contrast to the hematology analyzer as a way that can be taken to maximize examination without depending on experts, a tool was created that can be operated simply, in other words, an automatic hematology analyzer designed for experts without the need for special skills.

This hematology analyzer is designed as a tool that has easy-to-evaluate result accuracy because the accuracy and precision can be controlled, the number of cells counted is greater and the reading of the test sample only takes a short time to arrive at the desired result.

Yang explained that this method is the gold standard for measuring leukocyte concentrations as recommended by the International Committee for Standardization in Hematology (ICSH), so that a hematology analyzer can produce more valid results, because it has better sensitivity and specificity values.

CONCLUSION

Based on the results of this study it can be concluded that:

1. The results of leukocyte examination using a hematology analyzer were obtained13 normal results and 17 abnormal.
2. The results of leukocyte examination using a hemocytometer obtained 21 normal results and 9 abnormal results, 21 normal results and 9 abnormal results.

3. From the results of a comparison of leukocyte examination using a hematology analyzer and hemocytometer, it was obtained0.039 <0.05, where the null hypothesis (H0) means rejected and the alternative hypothesis (Ha) means accepted, so it can be concluded that the data obtained is different.

REFERENCES

[1] Afriona, 2020. Perbedaan Hasil Pemeriksaan Jumlah Leukosit Antara Metode Manual Improved Neubauer Dengan Metode Automatic Hematologi Analyzer, Di RSUD M. NATSIR SOLOK.

[2] Barbara J. 2014. Hematology Kurikulum Inti. Buku Kedokteran EGC. Jakarta.

[3] Devi,2016. Perbedaan Hasil Pemeriksaan Hitung Jumlah Leukosit Antara Metode Manual Improved Neubauer Dengan Metode Automatic Hematology Analyzer Pada Pasien Rawat Jalan Di Rsud Kota Kendari. *Karya Tulis Ilmiah.*

[4] Handayani, 2018. *Asuhan Keperawatan pada Klien Dengan Gangguan System Hematologi*, Selemba Medika : Jakarta.

[5] Jitowiyono, Sugeng., 2018. Asuhan keperawatan Pada Pasien Dengan Gangguan Sistem Hematologi. Pustaka Baru Press. Yogyakarta

[6] Keohane, 2016. *Rodak's Hematology Clinical Principles And Application.* Amerika: Elsevier.

[7] Kiswari, Rukman. 2014. Hematologi & Transfusi. Jakarta :Erlangga.Hal 3-5, 116- 120, 220-232

[8] Kosasih, A.S, dan Setiawan, L. 2016. *Panduan Pemeriksaan Hematologi.* Edisi I. PDS Patklin. Indonesia. Jakarta.

[9] Mujiburizal, M. N. F (2018). *Identifikasi Hitung Jumlah Leukosit Metode Manual Menggunakan Tabung Dengan Larutan Turk Dan Asam Cuka.* Malang; Stikes Maharani Malang.

[10] Mujiburizal, M. N. F (2018). *Identifikasi Hitung Jumlah Leukosit Metode Manual Menggunakan Tabung Dengan Larutan Turk Dan Asam Cuka.* Malang; Stikes Maharani Malang.

[11] Nugraha, Gilang. 2017 . Panduan Pemeriksaan Laboratorium Hematologi Dasar, Edisi II. Trans Info Media. Jakarta Timur

[12] Riswanto. 2013. Pemeriksaan Laboratorium Hematologi.Yogyakarta: Alfamedia & Kanal medika.Hal 1-6, 41-42, 72-80, 107-113.

[13] Sacher, R. A. 2012. *Tinjauan Klinis Hasil Pemeriksaan Laboratorium.* Jakarta: EGC

[14] *Word Heald Organization* (WHO), 2019. Weekly Epidemiological Recor.