ANALYSIS OF BLOOD SAMPLE LYSIS RATE ON HEMOGLOBIN EXAMINATION RESULTS USING RAYTO RT. 7600 AUTO HEMATOLOGY ANALYZER

Devi Cynthia Dewi, Adang Durachim
Master of Immunology, Post-graduate Program, Universitas Airlangga

ABSTRAK
Salah satu faktor yang paling banyak berpengaruh terhadap hasil pemeriksaan adalah preanalitik seperti darah yang lisis. Maka dari itu dilakukan penelitian tentang Analisis Tingkat Lisis Sampel Darah terhadap Hasil Pemeriksaan Hemoglobin. Penelitian itu dilakukan dengan menggunakan Alat "Rayto RT.7600 Auto Hematology Analyzer, dengan prinsip Flow Cytometri. Untuk membuat tingkatan lisis sampel darah maka sampel diberi perlakuan dengan menambahkan 0,5 ml darah ditambah 0,2 ml NaCl fisiologis (0,85%) untuk normal, NaCl 0,64% darah mulai lisis, NaCl 0,43% darah agak lisis, dan NaCl 0,21% darah lisis pada setiap perlakuan sampel darah. Setelah dilakukan penelitian hasil menunjukkan bahwa sampel darah normal rata-ratanya = 12,9333, pada sampel darah mulai lisis rata-ratanya = 11,890, pada sampel darah agak lisis rata-ratanya = 10,9000, dan pada sampel darah lisis rata-ratanya = 9,6633. Dengan menggunakan uji ANOVA ternyata penggunaan sampel darah yang lisis sangat berpengaruh terhadap hasil pemeriksaan, hal ini dapat dilihat dari nilai signifikansi dengan hasil nilai P (P-Value) lebih kecil (<) dibandingkan dengan nilai alpha (α) yang ditetapkan 0,05 (95%). Dari hasil tersebut maka dapat disimpulkan bahwa pada tingkat sampel darah agak lisis dan lisis tidak dapat digunakan untuk pemeriksaan Hemoglobin artinya penggunaan sampel darah yang lisis dapat berpengaruh terhadap hasil pemeriksaan Hemoglobin. (FMI 2014;50:262-264)

Kata kunci: hemoglobin, darah normal, lisis sampel darah

ABSTRACT
On internal quality assurance that is pre analytical laboratory, such as in laboratory area, particularly in hospitals, the research on the analysis of the blood sample lysis Level Examination Results Hemoglobin Using Tool "Rayto RT.7600 Auto Hematology Analyzer, the principle flow cytometric measurement method is the amount. To make the level of the blood sample lysis sample were treated by adding 0.5 ml of blood plus 0.2 ml physiological saline (0.85%) for normal, 0.64% NaCl starting blood lysis, NaCl 0.43% blood rather lysis, and 0.21% NaCl lysis of blood in each blood sample treatment. After doing research, the result showed that where normal blood sample mean = 12.9333, on blood sample began to lyse the mean = 11.890, on blood sample averaged somewhat lysis = 10.9000, and the lysis of blood sample averaged = 9.6633 and performed after statistical test by using ANOVA test, it can be seen in the statistics with ANOVA test, where P values obtained significance value (P-value) is smaller (<) compared with the value of alpha (α) set 0.05 (95%). From these results it can be concluded that the rate of blood sample rather lysis and lysis can not be used for examination of the use of Hemoglobin means that lysis of blood sample can affect the result of hemoglobin. (FMI 2014;50:262-264)

Keywords: hemoglobin, normal blood, blood lysis

Correspondence: Devi Cynthia Dewi, Master of Immunology, Post-graduate Program, Universitas Airlangga, Jalan Airangga 4-6, Surabaya, Indonesia. e-mail: devicynthia07@yahoo.co.id

INTRODUCTION
Hematology examination of is a set of laboratory tests which consisted of some types of examination, one of which is the examination of hemoglobin. The examination is a routine blood tests performed in the clinical laboratory, where the hemoglobin in a person can vary from one to another (Anonymous 1989). Hemoglobin is a protein compound with Fe (iron), called conjugation of proteins. The core is Fe with order protoporphyrin and globulin (tetra phyrin), where this hemoglobin that causes blood red due to Fe compounds. There are several ways that can be used to determine the levels of hemoglobin, among others Talquist, Sahli method, cupric sulfate, Cyanmethemoglobin method, and by using the tool Rayto RT.7600 Auto Hematology Analyzer. To determine the lysis rate of blood samples or whether it is affecting the results of the examination. This study used Rayto RT.7600 Auto Hematology Analyzer. The way the device works is by counting and measuring blood cells automatically by impedance variations in electric flow or light beam to the passing cells. This tool works on the principle of flow cytometer (Rayto 2011). In this study, blood hemoglobin levels were measured by using the tool "Rayto RT.7600 Auto Hematology Analyzer" (Rayto 2011). This study examined the results of hemoglobin in the blood sample with the level lysis rate and whether lytic blood
influenced on the results of hemoglobin by using the tool "Rayto RT.7600 Auto Hematology Analyzer".

MATERIALS AND METHODS

This research was an experiment that is conducted in the laboratory that produces the data and reveal the work steps and performance parameters of analytical methods. According to Gomez (1995), the number of samples to be taken from the population is 10 samples. Design The study compared the data group hemoglobin by using the tool "Rayto RT.7600 Auto Hematology Analyzer", and the results calculated using ANOVA statistical test. Populations comprised entire student of Health Analyst Academy Harapan Bangsa Bengkulu. Samples: 10 students of Health Analyst Academy Harapan Bangsa Bengkulu. The study was conducted at the Laboratory of Hematology, Health Analyst Academy, Harapan Bangsa, Bengkulu. The study was conducted in 19 August - 4 September 2013. The tools used in the study; syringe, toniquet, reaction tube, micropipette, Rayto RT.7600 Auto Hematology Analyzer, markers, and pipette measure.

Reagents were diluent, rinse, lyse, probe cleanser, and EZ cleanser, K3EDTA (Etylene diamine Tetra Acetate), 50 ml physiological saline. Materials used in the study: Venous blood was 3 ml. Hemoglobin examination (by using the tool "Rayto RT.7600 Auto Hematology Analyzer". Four pieces of test tube were taken and then entered into each tube 85% physiological saline 0.2 ml, NaCl 0.64% 0.2 ml, 0.2 ml 0.43% NaCl, 0.21% NaCl and 0.2 ml for each treatment. Then, blood contained in EDTA Vacutainer tubes 0.5 ml was added to each reaction tube in each treatment, and then check by using the "Rayto RT.7600 Auto Hematology Analyzer".

Hematology analyzer mode: Press the ON/OFF button on the rear of the tool "Rayto RT.7600 Auto Hematology Analyzer" and then wait for initializing process is completed. The running of samples procedures for whole blood samples is as follows: Press the "Menu" and select "Sample Mode" and then press the "Enter", select "Whole Blood" and then press the "Main", press the "ID" to enter the patient's ID number and then press "Enter" button, press the button "Open" to open the door of a sample, a sample rotate in position 1 for Whole Blood, EDTA Vacutainer tubes containing blood samples had been placed on the first roller machine until a homogeneous mixed blood then inserted into the tube samples, close the door and pressing the "aspirate", the results can be seen on the monitor screen tool Rayto RT.7600 Auto Hematology Analyzer. The steps above are repeated for the next sample.

RESULTS

From the results of Anova Test significant value (sig) revealed p value = 0.000, less than a real level of 0.05, meaning that there is a difference of treatment on blood samples lysis. To see the difference we carried out Least Significant Difference (LSD) test. From the results of LSD test significant value (sig) were less than 0.05 is 0.000, it is proved that the blood samples were lysis the results of the examination.

<table>
<thead>
<tr>
<th>(I) Lysis rate</th>
<th>(J) Lysis rate</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1.04333*</td>
<td>.15433</td>
<td>.000</td>
<td>.7377</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>-1.04333*</td>
<td>.15433</td>
<td>.000</td>
<td>-1.3490</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>-2.9900*</td>
<td>.15433</td>
<td>.000</td>
<td>-1.2957</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-2.03333*</td>
<td>.15433</td>
<td>.000</td>
<td>-2.3390</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-3.22667*</td>
<td>.15433</td>
<td>.000</td>
<td>-2.9210</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.23667*</td>
<td>.15433</td>
<td>.000</td>
<td>.9310</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-1.23667*</td>
<td>.15433</td>
<td>.000</td>
<td>-1.5423</td>
</tr>
</tbody>
</table>

The mean difference is significant at the 0.05 level.
DISCUSSION

In blood samples treatment physiological saline was diluted again to 0.64% NaCl, 0.43% NaCl and 0.21% NaCl, thus becoming hypotonic solution, because WP (water potential) of NaCl solution was higher than the WP of red blood cells. Sometimes a particular concentration of NaCl solution does not experience erythrocyte hemolysis experienced. In old erythrocyte cell membranes have a low tolerance (easily broken), while in young erythrocyte, the membranes have a higher osmotic tolerance (not broken).

By using the tool Rayto RT.7600 Auto Hematology Analyzer hemoglobin levels starts to decrease in blood samples that began to lysis, nearly lysis of completely lysis. This is because the working principle of the appliance Rayto RT.7600 Auto Hematology Analyzer is based on the principles of flow cytometer, so if the cells had been lysed erythrocytes, hemoglobin and erythrocytes which are outside, which had been broken, cannot be measured by the instrument. The one measured/read by the tool is hemoglobin in the cells which are intact erythrocytes/without lysis.

Level difference lysis of blood samples is due to the level of blood samples began lysis, a small portion of erythrocytes rupture. Heme and globin and only a few are outside the erythrocytes, so that when measured with a Rayto RT.7600 Auto Hematology Analyzer the results are within normal limits. It is because that the measured one is hemoglobin that still resides in erythrocytes were still intact and not lysed. At the level of nearly lysis blood sample, most of the erythrocytes rupture, so the hem and globin just mostly located outside of erythrocytes, and by the time measured with a Rayto RT.7600 Auto Hematology Analyzer the results are below the normal value and this is because the measured one was hemoglobin residing in erythrocytes were still intact and not lysed. At the level of a blood sample lysis, many erythrocytes were broken, so many heme and globin were outside the erythrocytes, and by the time measured with a "Rayto RT.7600 Auto Hematology Analyzer", the results were below the normal value, as measured is hemoglobin that is still being in erythrocytes were still intact and not lyse. This was because the tool Rayto RT.7600 Auto Hematology Analyzer works on the principle of flow cytometry.

CONCLUSION

Nearly lytic and completely lytic blood affects the result of examination, and the use of lytic blood sample affects the results of hemoglobin examination.

REFERENCES