

Original Articles

Comparison of Automated and Manual Reticulocyte Count Determination

Viroj Wiwanitkit

Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330

Objective: To compare automated and manual reticulocyte count determination. **Study Design:** Descriptive study. **Setting:** Faculty of Medicine, Chulalongkorn University. **Methods:** Eighty blood samples from both inactive and active erythropoiesis states were collected for the comparative study of reticulocyte count determination. All samples were determined by two methods-manual and automated reticulocyte count. The technicians who did the test were blinded for the other method results. Average values from reticulocyte count determination were calculated, compared then analyzed. **Results:** The average reticulocyte count values by manual method was $2.89 \pm 2.59\%$. The average reticulocyte count values by automated method was $2.24 \pm 2.30\%$. It revealed that there was no significant difference between reticulocyte count values determined by both methods ($p > 0.05$). **Conclusion:** The reticulocyte count results from automated and semi-automated methods are not significant different. The manual reticulocyte count in routine service is recommended.

Key Words : ● Reticulocyte count

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The reticulocyte count is one of the most valuable test to assess the bone marrow erythropoietic activity¹. In the present day, there are two common methods to perform reticulocyte count, manual and automated reticulocyte count. Manual reticulocyte count is basic com-

mon laboratory method for determination of the number of reticulocyte. Automated method makes use of automated hematology machine to directly perform reticulocyte count². Presently, recent technological advances led the way to development of blood cell analyzer capable of performing a complete analysis of circulating reticulocytes and erythrocytes.

Due to the fact that interpretation of such hematological parameters must base on the

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Requests for reprints should be addressed to Dr. Viroj Wiwanitkit, M.D., Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330 Thailand.

references and quality of the tests so much, therefore, in this study, a comparison between the two methods was performed. Results from this study can be useful data for the physicians in interpretation of laboratory results and in selection of the laboratory tests.

Materials and Methods

This study was carried out in Division of Laboratory Medicine, King Chulalongkorn Memorial Hospital during June 2000. Eighty blood samples from both inactive and active (as from the thalassemic patient) erythropoiesis states were collected for the comparative study. All blood sample was collected after informed consent to the subject and 5 milliliter of EDTA blood sample from antecubital venipuncture was used.

The comparative study of determination of reticulocyte count by manual and automated methods was performed. Two sets of practitioners were assigned for the two methods. The technicians who did the test were blinded for the other method results. The manual reticulocyte count in this study was performed using dry supravital stain method³. Automated reticulocyte count in this study was performed using the automated hematology analyzer, Technicon-H1. The Technicon-H1 is an automated blood cell analyzer that performed CBC and reticulocyte count using an optical method based on the measurement of scatter and absorption of helium-neon laser light. The automated reticulocyte count method requires a preliminary

manual mixing of 3 microliter of blood sample with 3 milliliter of reticulocyte reagent, containing a surfactant, which sphere red blood cells and reticulocytes, and the nucleic acid-binding dye oxazine 750, which selectively stains reticulocytes by complexation with cytoplasmic RNA. After a 15-minute incubation, the prepared sample is aspirated through the automated analyzer flow cell, where three detectors measure laser scatter, at low angle (2-3 degree) and high angle (5-15 degree), and absorption. On a two-dimensional cytogram of absorption versus low angle scatter, the stained reticulocytes are separated from unstained erythrocytes, platelets, and leukocytes by appropriate thresholds. Descriptive statistical analysis was performed where it was appropriate. Comparison was performed using two-tailed paired T-test. Correlation coefficients were also performed to assess significant differences in determination of reticulocyte count by manual and automated methods. p-value equaled to 0.05 was accepted as significant level.

Results

The average reticulocyte count values by manual method was $2.89 \pm 2.59\%$. The average reticulocyte count values by automated method was $2.24 \pm 2.30\%$. It revealed that there was no significant difference between reticulocyte count values determined by both methods ($p > 0.05$). Comparing determination by manual to automated methods gave correlation coefficient = 0.903 for reticulocyte count (Figure 1).

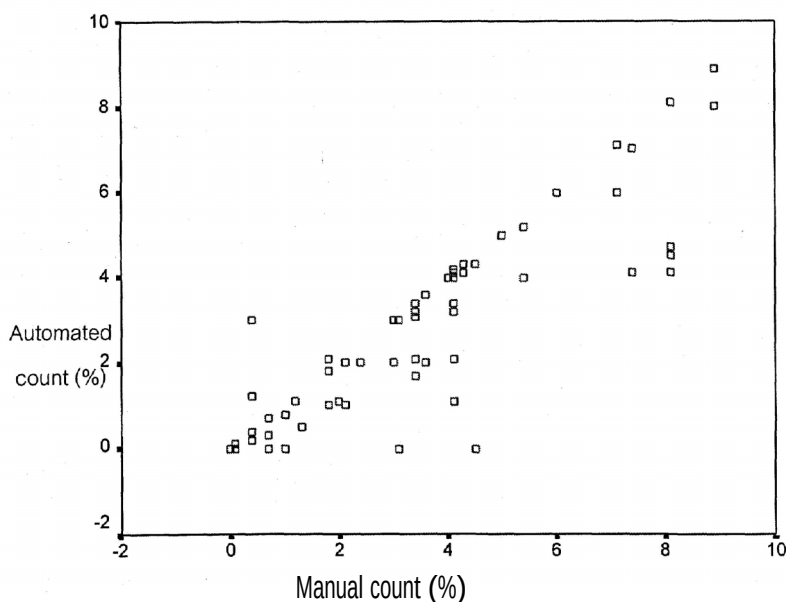


Figure 1 Reticulocyte count value from manual and automated methods

Discussion

Under physiological condition, the reticulocytes mature in the bone marrow for about three days and subsequently in the blood circulation for an additional day until they become mature red blood cell. The reticulocyte is an important hematology test for monitoring of erythropoiesis.

In this study, a comparison between manual and automated methods for determination of reticulocyte counts was performed. It revealed that there was no significant difference between both methods. Therefore, whatever methods used in determination of reticulocyte counts, it seems to have no effect in the interpretation of the laboratory results on the general references⁴.

The manual method using supravital stain has its limitations for example the variable distribution of reticulocytes in the blood smear,

the small number of reticulocytes actually counted. In addition, technicians widely vary as to morphological identification and enumeration of reticulocytes. Furthermore, it also time-consuming. But it is a cheap test and can performed as bedside test⁵.

Concerning to previous study⁶, the automated method for determination assay can be the candi-

date for conventional manual clotting assay technique. The automated reticulocyte method can provide faster turnaround time and it can provide other useful reticulocyte parameters. But considering details, the instrument cost of automated hematology machine is very high and it seems not to cost-effective in small setting⁷. From this study, it seems that manual reticulocyte is better test for routine reticulocyte count, especially for the rural hospital setting. While the automated reticulocyte seems to be an effective tool in advanced hematology and research center than using as the routine laboratory test.

But the important fact that should be concerned is all physicians should realize that not only laboratory but also data from physical examination and history taking must be combined in diagnosis of patient hematological problems.

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นิพนธ์ต้นฉบับ

การศึกษาเปรียบเทียบการหาค่า Reticulocyte ด้วยวิธีใช้บุคลากรและเครื่องตรวจอัตโนมัติ

วิโรจน์ ไวนิชกิจ

ภาควิชาเวชศาสตร์ชั้นสูติฯ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330

วัตถุประสงค์: เพื่อเปรียบเทียบการหาค่าของ reticulocyte count ด้วยวิธีใช้บุคลากรและวิธีอัตโนมัติ **รูปแบบ:** การศึกษาเชิงพรรณนา **สถานที่ศึกษา:** คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **วิธีการ:** ได้เก็บตัวอย่างเลือดจำนวน 80 ตัวอย่างจากกลุ่มผู้ป่วยที่มีสภาวะของการสร้างเม็ดเลือดแดงในภาวะมีและไม่มีภาวะกระตุ้นการสร้าง โดยได้หาค่า reticulocyte count สำหรับแต่ละตัวอย่างโดยได้ทำการทดสอบแต่ละตัวอย่างด้วยวิธีใช้บุคลากรและเครื่องตรวจอัตโนมัติ โดยบุคลากรที่ตรวจด้วยวิธีหนึ่งจะไม่ทราบผลของอีกวิธีขณะตรวจ นำค่าเฉลี่ยที่ได้มาเปรียบเทียบและวิเคราะห์ **ผลการศึกษา:** จากการศึกษาได้ค่าเฉลี่ยของ reticulocyte count สำหรับวิธีใช้บุคลากรและวิธีอัตโนมัติ วิธีใช้บุคลากรและวิธีอัตโนมัติ เท่ากับ ร้อยละ 2.89 ± 2.59 และ 2.24 ± 2.30 ตามลำดับ ทั้งนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างวิธีการตรวจทั้งสองวิธี **บทสรุป:** จากการศึกษาพบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างวิธีการตรวจทั้งสองวิธี ทั้งนี้ได้อภิปรายข้อแนะนำในการใช้การตรวจด้วยวิธีใช้บุคลากรซึ่งเหมาะสำหรับการตรวจตามปกติโดยทั่วไป

Key Words : ● Reticulocyte count

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2544;11:37-40.