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Manual and Automated Comparatıve Analysıs in Retıculocyte Countıng Method

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Abstract: The traditional method of reticulocyte count is based on microscopic evaluation of peripheral blood films. Automated processes have replaced the manual method of reticulocyte counts in most laboratories. They are readily available and give more precise results. The present study was conducted to compare the manual and automated methods of reticulocyte estimation. The study was conducted for six months, from January to June 2019, at a tertiary care center in Dibrugarh, Assam. One hundred twenty-six venous blood samples of patients were analyzed, of whom 27 were males and 99 females. Student t-test between the two methods showed a p-value of 0.41. Pearson's coefficient between the two methods showed r value= 0.992. Scatter plot and Passing & Bablock regression analysis showed a positive correlation between the two methods. The present study showed a good correlation between manual and automated methods. The manual method can use the manual method of counting reticulocytes in situations of limited cost. In contrast, the automatic method can be an option when fast results and large sample sizes are required.
Keyword: Reticulocyte; supravital stain; automated method.

INTRODUCTION

Reticulocytes are immature red cells containing remnants of the ribosomal ribonucleic acid (rRNA). A characteristic property of ribosomes is to react with certain basic dyes such as azure B, brilliant cresyl blue, or new methylene blue that forms a blue or purple precipitate of granules or filaments. This reaction takes place only in vitally stained unfixed preparations. (Bain B, 2017)The reticulocyte count is commonly used as an indicator of the erythropoietic activity of the bone marrow. It is essential for the diagnosis, classification, and monitoring of the treatment of anemias. It also aids in the follow-up of the bone marrow regeneration after intensive chemotherapy, bone marrow transplantation, and monitoring the response of erythropoietin therapy in chronic renal failure (Maconi M et al. 2010).

Both manual and automated methods can do a reticulocyte count. The traditional manual method of counting reticulocytes under light microscopy was developed in 1940 and is based on the property whereby the supravital stain precipitates the remaining portions of ribosomal RNA.(Pierre RV 2002, Buttarello M et al, 2001, Riley RS et al, 2002)

In the automated method of reticulocyte count, a fluorochrome is used for staining the remnant ribonucleic acid (RNA) present at the reticulocyte. After being

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dyed, the fluorescent cells can be enumerated using a flow cytometer of general use. (Bain, B. J.2004).

Research comparing reticulocyte count results on automatic and manual tools has been carried out as Gorte 2020 studied with the Horiba Pentra XLR automated tool. However, each automatic device will have different results values because of unique fluorescent dye variations in the device used. So this study aims to compare the results of manual reticulocyte counting with the automatic method using Sysmex XN550.

MATERIALS AND METHODS

The present study was conducted in a tertiary care center in Dibrugarh, Assam. It was a hospital-based cross-sectional study. The duration of the study was six months, from January to June 2019. We collected 126 blood samples for which reticulocyte count was requested. This study follows research ethics rules based on the COPE and Declaration of Helsinki. Informed consent was obtained from the patients or the guardians in the case of the pediatric age group. The pediatric population range was from 0 (newborn) to 10 years, and the adult population range was from 15 to 75 years. There were 27 males and 99 females. All blood samples were collected in tripotassium EDTA vials and were stored at room temperature. The samples were analyzed within 6 hours of collection.

Manual Method

2-3 drops of Brilliant Cresyl Blue solution in a test tube plus the same amount of blood mixed thoroughly. The mixture was stored at 37°C for 15-20 minutes. The red blood cells were resuspended by gentle mixing, and a peripheral blood film was prepared on a glass slide. After drying, the blood films were examined without fixing or counterstaining. Light microscopy was used to count reticulocytes in 1000 erythrocytes per smear from the sample. Experts in the hospital laboratory carry out the calculations. The result is expressed as the percentage of cells containing reticulocytes.

Automated method

Blood samples were analyzed using a Sysmex XN550 analyzer. The Sysmex XN550 is a fully automated 6 part differential hematology analyzer. The principle of reticulocyte estimation under automated methodology is based on fluorescence flow cytometry. The instrument was used as per the manufacturer's instructions. Results of the reticulocyte counts were expressed in percentages. The device was calibrated according to the manufacturer's instructions, and quality control was run daily.

Statistical Analysis

Statistical analyses were done using the student's t-test. A p-value <0.05 was considered statistically significant. The correlation coefficient was calculated by Pearson's method for determining the strength of association between the two methods. A linear relationship was studied through a scatter plot. Passing and Bablok did agree between the methods. This equation states no statistical difference between the reference and test method if respective confidence intervals include a slope of (B1) 1 and intercept (B0) of zero, indicating the absence of absolute and proportional bias, respectively (Passing H, 1983).

RESULTS AND DISCUSSION

A total of 126 samples were included in the study. From 126 samples, there were 27 (21.4%) males and 99 (78.5%) females. The age group of patients ranged

from newborn to 75 years with a mean age of 28.4 yrs. Manual reticulocyte results ranged from 0.5 to 24%, while automated results ranged from 0.47 to 25.7%.

Table 1. Student's T-test Statistic Results

	Mean	Standard deviation	p-value
Manual	3.63	4.20	0.41
Automated	4.07	4.58	

Table 1 shows the student's t-test with the mean and standard deviation of both methods. P-value was 0.41. Therefore, it cannot be concluded that a significant difference exists between the two ways.

Table 2. Correlation Coefficient for Reticulocyte Enumeration

	Manual vs Automated
r	0.992
p-value	0.000

Pearson's method was used to calculate the coefficient of correlation between the two ways. The correlation between the two methods is excellent; r=0.992. Correlation between the two methods is also statistically significant: p<0.000

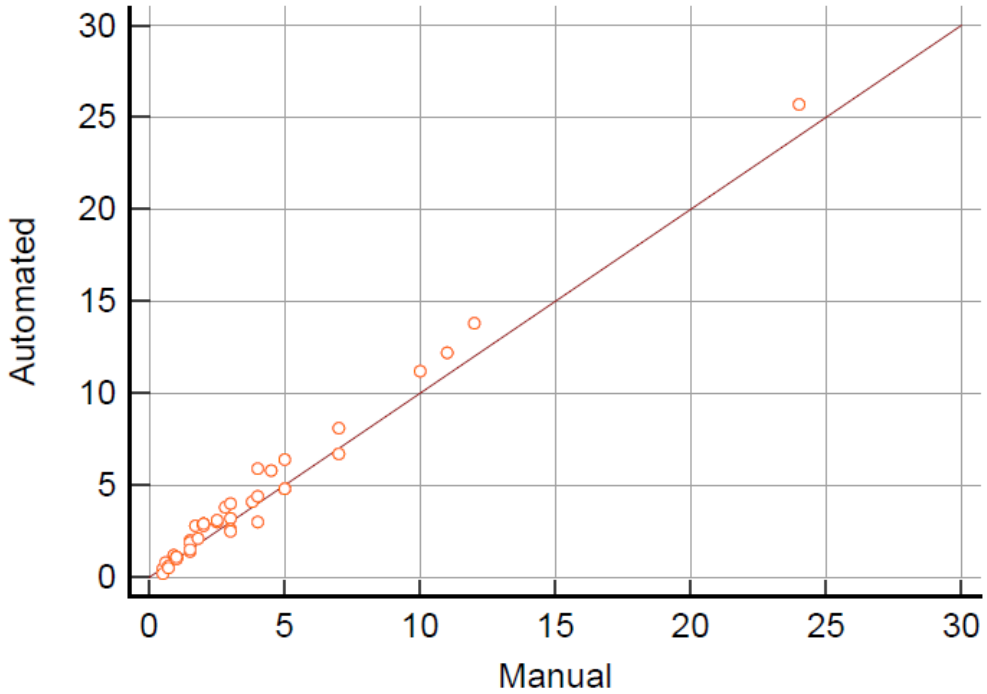

Figure 1: Scatter Plot for Data of Reticulocyte Count (%) of Manual and Automated Methods

Figure 1 shows the linearity between manual and automated methods using the scatter plot. In the above scatter plot, it can be seen that the data points of both methods follow almost a straight line. Hence, it can be inferred that there is a positive correlation between the two ways.

Table 3: Comparison of the Two Methods by Passing & Block Linear Regression Equation

	Manual vs. Automated
Intercept B0	-0.069
95% confidence interval	-0.175 to -0.010
Slope B1	1.14
95% confidence interval	1.11 to 1.20

The table above shows the intercept value as -0.069 and slope value as 1.14. Regression analysis by Passing and Block indicates not enough evidence to conclude that the methods are not equal.

Reticulocyte count reflects the rate of erythropoiesis in the bone marrow. It is a commonly requested test by most clinicians to evaluate patients with anemia. Therefore, an accurate estimation of the reticulocyte count plays a critical role in the diagnosis and monitoring of the treatment of patients.

In this study, we performed a comparison between manual and automated methods for reticulocyte counting. The present study showed a good correlation between manual and automated methods. Our result is comparable with Gorte et al. They found no significant difference between automatic and manual methods for reticulocyte counting. A significant positive correlation was found between the two methods using Pearson's correlation coefficient (r=0.985, p=0.0001). (Gorte et al., 2020)

Similar results were obtained by Simionatto et al. They showed that the difference between the two methods was minimal, with an estimated 0.4% systematic error and 3.9% random error. Thus, it has been confirmed that both methods, when well conducted, can reflect precisely the reticulocyte counts for good clinical use. (Simionatto et al., 2010)

Nobes PR et al. showed that the overall correlation between flow cytometry and manual methods is excellent, giving a correlation coefficient of 0.99 with slope 0-96 and intercept 0-02. (Nobes PR et al.,1990)

Ferguson DJ et al. found that linearity was highly acceptable (r = 0.99) over the reticulocyte count range of 1.8-30.1% (Ferguson DJ et al., 1990)

Riley et al. showed that comparing the reticulocyte counting on flow cytometry with the manual methodology resulted in an excellent correlation (R=0.984). (Riley et al.,2002). Ali et al. (2010) found a high degree of correlation and excellent agreement between the two methods. Our study in table 2 also showed a good correlation (R=0.992) between the two methods.

The reticulocyte count can be done manually or automatically because, from the research results, these two methods have good correlation results. Manual reticulocyte counting has been the standard method since 1940 and is a simple method with low cost because it only uses a microscope (Piva E et al., 2010). The manual method has limitations such as more time required for analysis, lack of stain quality, and inappropriate blood films (Viana KA et al., 2014) While the automatic reticulocyte count uses a faster and more sensitive time for diagnosis and therapeutic monitoring of anemic patients (Davis BH et al., 1994). In addition, automatic reticulocyte counting is more specific and efficient (Torino AB et al., 2015).

The limitation of this study is respondents doesn't divide between anemic and not anemic condition patient. Whereas the possibility of anemic blood samples will affect the results of the examination, such as macrocytic anemia or hemolytic anemia.

CONCLUSION

Our study concludes that the variation between the two methods is very low. A linear relationship was obtained between the two methods for both lower and higher reticulocyte counts. We, therefore, recommend proper implementation of both methods to get appropriate results to assist clinical diagnosis, treatment, and patient monitoring.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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