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IP Journal of Diagnostic Pathology and Oncology

Journal homepage: https://www.jdpo.org/

Original Research Article

Manual versus automated method for reticulocyte count: A comparative study

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ARTICLE INFO

Article history:
Received 01-12-2023
Accepted 27-01-2024
Available online 31-01-2024

Keywords:
Reticulocyte count
Supravital dye
Manual method

ABSTRACT

Aims and Objective: To compare the reliability and degree of acceptability of manual reticulocyte count over automated method in Tertiary Care Hospital

Background: Reticulocytes are precursory to erythrocyte cells released from bone marrow into the blood and their evaluation is helpful in early diagnosis as well as therapeutic monitoring of anemic patients. Reticulocyte count is a rapid and basic hematological test that measures the erythropoietic activity within the bone marrow.

Materials and Methods: An analytical and observational hospital-based study was conducted on all EDTA blood samples received for reticulocyte count by both manual and automated methods. A comparison of both methodologies was done.

Results: An evaluation of 230 blood samples was conducted and it showed a strong correlation between both methodologies with a p-value more than 0.05.

Conclusion: The study suggests that the manual method for reticulocyte count is as reliable as an automated method. Its cost-effectiveness and reliability are useful in small urban laboratories and also in remote rural areas for early diagnosis as well as treatment of anemia.

1. Introduction

Reticulocytes are precursory to the erythrocyte cells in the blood that are released from bone marrow containing remnants of ribonucleic acid (RNA) and ribosomes but no nucleus.[1] They lose RNA a day after reaching blood from bone marrow but continue to synthesize hemoglobin even after the loss of the nucleus.[2] The reticulocytes, when in bone marrow take 1-3 days for development whereas when in blood, they have a life span of 1-2 days before becoming fully matured RBCs.[3]

Reticulocyte count (RC) is a rapid and basic hematological test used to measure the index of erythropoietic activity within the bone marrow[4] for initial assessment of anemia and in providing vital parameters helpful for a sensitive approach to the diagnosis and therapeutic monitoring of the anemic patients.

Increased erythropoiesis depicted by reticulocytosis develops in response to various conditions like hemolytic anemia, blood loss, post-therapy for nutritional anemia and hemoglobinopathies. On the contrary, decreased erythropoiesis is represented by reticulocytopenia due to renal disease, anemia of chronic disease, alcoholism, bone marrow failure, blood transfusion, aplastic anemia and pure cell aplasia.[1]

The clinical laboratories currently operate by two methods for reticulocyte counting. One being the traditionally used manual method and the other being the automated method.[5] With the advent of automation, the precision of reticulocyte count has remarkably improved.[6] The use of automated analyzers has recently increased

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https://doi.org/10.18231/j.jdpo.2024.008

replacing the manual/ visual method of reticulocyte count in the urban-based as well as in heavily loaded laboratories. But the visual method is still in use in resourced strained laboratories[7] more specifically in rural areas.

1.1. Normal range

The normal range by both the methodologies for adults is 0.5 – 2.5 % and for newborn infants is 2.5 – 6.5 % (which by the second week of life is the same as that of adults)[8].

This study aims to compare manual and automated methods for reticulocyte count and evaluate the degree of acceptability of manual reticulocyte count in terms of accuracy and cost.

2. Materials and Methods

An analytical and observational hospital-based study of the manual as well as automated reticulocyte count was conducted on blood samples received over a period of 1 year (June 2022 - 2023) at the Department of Pathology of Tertiary Care Hospital after getting clearance from the Ethics Committee of the Institute.

All the venous blood samples collected in EDTA vials were received for reticulocyte count and processed within 4 hours of collection by manual as well as automated methods. The clinical details were taken from clinical records.

Comparison of both methods was done for each blood sample.

2.1. Statistical analysis

All the statistical analysis was done with the help of the SSPS (Statistical Package for the Social Sciences) software system. Descriptive statistics of the outcome of reticulocyte count by automated and manual methods were done by summarizing them as mean with standard deviation and conducting paired t-tests to compare the statistical results.

If the P-value < 0.05, then it was considered a significant result.

In this study, a total of 230 blood samples of patients with anemia were taken for comparison of manual and automated methods of reticulocyte count.

2.2. Manual method

Principle: Reticulocytes when stained with a supravital dye, precipitate the RNA to form a dye-ribonucleoprotein complex[2] which appears as a pale blue reticulocyte containing dark-blue network/granules under microscopic examination.

The supravital dyes that can be used are new methylene blue, brilliant cresyl blue, crystal violet and methyl violet.[9] For this study, we used new methylene blue for the staining procedure by manual method.

Procedure: Three drops of blood and three drops of the reagent (supravital dye) were taken in a test tube, mixed, and incubated for 15 minutes at room temperature. Two thin wedge films were made from this mixture and air-dried. Using a compound microscope, counting of 1000 red cells was performed under a x100 objective lens in oil immersion.[2] Reticulocytes appear as pale green-blue stained cells containing dark blue-violet clumps or granules while mature red blood cells appear as pale green-blue colour without clumps.

The counting of reticulocytes by manual method is based on the intensity of staining of cells and is divided into stages of maturation given by Heilmeyer.[1]

1. Stage 0 - Late or orthochromatic normoblast.
2. Stage **I** – Dense cohesive reticulum in non-nucleated red cell.
3. Stage **II** – Extensive network of loose reticulum.
4. Stage **III** – Small reticulum along with scattered granules
5. Stage IV – Scattered granules.

Stage 0 is not taken into consideration while counting the cells manually.

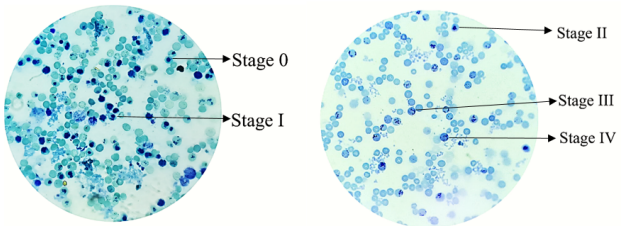

Figure 1: Different stages of maturation of reticulocytes under x100 oil immersion magnification view

2.3. Measurements of reticulocytes:[10]

1. Reticulocyte count – It is the number of reticulocytes amongst 1000 RBCs and is expressed in percentage
   Reticulocyte percentage = No. of reticulocyte/ Total no. RBC x 100
2. Corrected reticulocyte count – It is reticulocyte count corrected for evaluation of degree of anemia.
   Corrected reticulocyte = % reticulocyte x (Patient Hct/45)
3. Absolute reticulocyte count – It is the number of reticulocytes in 1 cumm of blood
   Absolute reticulocyte count = % Reticulocyte x RBC count/L^3

2.4. Automated method

Principle: The RNA remnants in immature erythrocytes are stained with fluorescent dye by penetrating the cell

membranes. Reticulocytes are measured on the principle of forward light scatter. The fluorescence-stained reticulocytes and erythrocytes are divided into 4 fractions by the intensity of fluorescence – HFR, MFR, LFR, and RBC.[11]

The instrument used in this study was SYSMEX XN 550, a fully automated instrument that uses polymethine dye for analysis through fluorescence and light scattering methods. It provides parameters like RET%, IRF, Ret-He, RET#, RPI.[11]

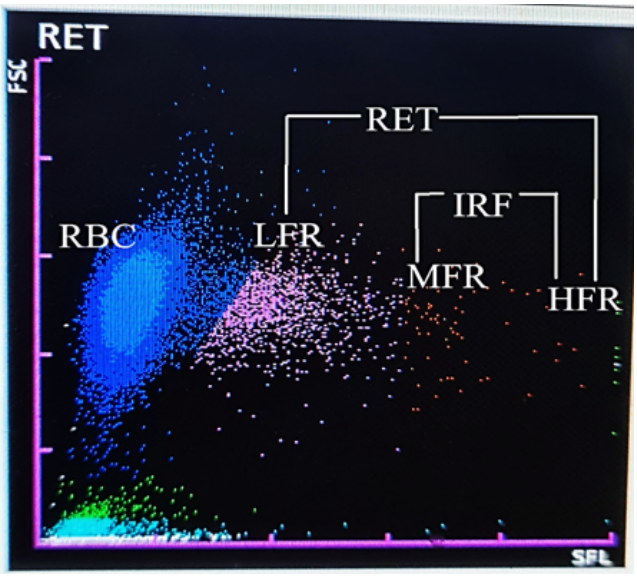

Figure 2: Scattergram for reticulocyte on Sysmex XN 550

3. Results

The demographic data in the study period involved a total of 230 cases for evaluation of reticulocyte count by manual and automated methods, of which 120 cases were male and 110 females [Table 1]. Among these, 17 cases were infants (age < 1 year), 47 were children (aged between 1 -14 years) and 166 were adults (individuals above the age of 14 years)[Table 2].

Table 1: Gender distribution

Total number of cases (n)	230	Percentage
Male	120	52.17%
Female	110	47.82%

Table 2: Age distribution

Total number of cases (n)	230	Percentage
Infants (<1 year)	17	7.39 %
Children (1-14 years)	47	20.43 %
Adults (>14 years)	166	72.17 %

The gender-based comparison showed the mean reticulocyte count for males by automated as well as for manual methods to be 4.69 ± 4.20 and 4.79 ± 4.36 respectively. For females, the mean reticulocyte count by automated method was 3.65 ± 3.52 and manual method by 3.78 ± 3.63. The statistical analysis showed no significant difference between the two methods as the P value for males was 0.75 and for females 0.62, both of which are more than 0.05 [Table 3].

Table 3: Comparison of reticulocyte count as per gender distribution

Gender(n = 230)	Mean automated reticulocyte count (± SD)	Mean manual reticulocyte count (± SD)	P Value
Male (n = 120)	4.69 ± 4.20	4.79 ± 4.36	0.75
Female (n = 110)	3.65 ± 3.52	3.78 ± 3.63	0.62

A comparison of reticulocyte count by both methods was also done based on the morphological classification of anemia in both genders [Table 4]. The study showed a prevalence of normocytic and macrocytic anemia in males, while microcytic anemia was common in females. However, the statistical difference between the mean automated and manual reticulocyte count based on morphological classification was found to be insignificant in respective gender groups.

Table 4: Comparison of Reticulocyte count according to morphologic classification of anemia

Types of anemia (n = 230)	Mean automated reticulocyte count (± SD)	Mean manualreticulocyte count (± SD)	P Value
Microcytic anemia (n = 135)			
Male : 70	4.64 ± 4.22	4.68 ± 4.45	0.81
Female: 65	3.29 ± 2.80	3.68 ± 2.90	0.59
Normocytic anemia (n = 71)			
Male : 35	4.41 ± 3.73	4.61 ± 3.92	0.78
Female : 36	3.70 ± 3.25	3.75 ± 3.01	0.92
Macrocytic anemia (n = 24)			
Male : 13	5.80 ± 5.41	5.91 ± 5.39	0.93
Female : 11	5.37 ± 6.12	5.56 ± 6.89	0.97

4. Discussion

The demographic data showed a prevalence of anemia in males as compared to females. This study had no statistical difference between the mean reticulocyte count in both the methodologies among males as well females showing a reliability over the manual method similar to that of the automated method. The value which was high by one method was also high when done by the other method.

The study showed a high degree of correlation between visual and automated methods which was consistent with the studies previously done by Gorte TR et al (2020)[12] and Viana, Karina & Filho et al (2014).[13]

This study was only focused on the difference between the automated and manual methods for reticulocyte counting and observed no difference in values obtained by both the methodologies in all microcytic, normocytic and macrocytic types of anemia. The highest mean reticulocyte count by both automated method and manual method was observed in males with macrocytic type of anemia. Whereas, the lowest mean reticulocyte count was seen in microcytic anemia in females.

Some literatures also found significant differences in their studies.[14,15] The reported differences in values may reflect due to various underlying factors like lack of staining quality**,** inappropriate techniques, inappropriate counting and calculations, faulty blood films or slides or delayed evaluations. In this study, precautionary measures were taken to minimize the erroneous results. The samples were processed within 4 hours of collection by trained technicians with the proper use of only one type of quality dye for staining (new methylene blue).

5. Highlights & Challenges

Both methodologies have their benefits and drawbacks. The automated method analyses a higher number of samples in one go, utilizes a large number of cells for counting and provides individual cell characteristics with various parameters at a single time point. The method though rapid and precise, can be expensive for small-scale laboratories with a smaller number of samples per day.

The manual method on the other hand has low reproducibility, evaluates a smaller number of cells while counting, is laborious, time consuming, requires skills for reporting and relies on visual acuity and patience of the observer. But it can be cost-effective for small laboratories as well as economical from patients' point of view.

Various interferences can also affect the results of an automated method for reticulocyte count like giant platelets, platelet clumps, abnormal WBCs, fragmented WBCs, nucleated RBCs, intraerythrocytic particles (Howell-jolly bodies, basophilic stippling, Heinz bodies etc.).[11] These challenges can be ruled out by visual methods and can play a pivotal role in the clinical investigation of the degree of ineffective erythropoiesis.

6. Limitations

1. In our study, the demographic data was restricted to only one hospital in the area and hence the study population was less.
2. Further, a study should be developed considering each disease condition separately where reticulocyte count is requested.

7. Conclusion

1. Both the automated and manual methods provide similar results for the enumeration of reticulocytes with the use of standard operating protocols and proper sample handling by skilled personnel. The study concluded that the manual method for reticulocyte count is as reliable as an automated method.
2. The automated method being rapid with the provision of more parameters is expensive having a restriction of usage in only large-scale clinical laboratories and tertiary care centers.
3. The manual method's cost-effectiveness, simplicity and reliability is useful and can be depended upon in small-scale urban laboratories and also in remote rural areas for early diagnosis as well as treatment of anemia.
4. This study also emphasizes the practice of manual method for reticulocyte count in areas where automated method is not accessible for the improvement of healthcare quality, as a simple basic test can be of paramount importance in diagnosing and treating various clinical conditions.

8. Abbreviations

RBC - Red cell count, HFR – High fluorescence ratio, MFR – Medium fluorescence ratio, LFR – Low fluorescence ratio, IRF – Immature reticulocyte fraction, Ret-He – Reticulocyte hemoglobin equivalent, RET# - Reticulocyte fraction, RPI – Reticulocyte production index

9. Source of Funding

None.

10. Conflict of Interest

None.

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Cite this article: Singh M, Mane VP. Manual versus automated method for reticulocyte count: A comparative study. *IP J Diagn Pathol Oncol* 2024;9(1):44-48.