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Comparison of Automated and Manual Reticulocyte Count in a cohort of patient's samples in Haematology Laboratory of Colombo South Teaching Hospital, Sri Lanka

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Abstract

Background: The reticulocyte count represents the marrow erythroid activity and it is a very important diagnostic tool in disorders of erythropoiesis. Reticulocyte count estimation is performed manually or using automated analyzers, depending on the laboratory facilities. This study aims to compare automated and manual reticulocyte count methods and to determine, whether there is a significant variance between the results of the two techniques.

Materials and method: Sixty-five EDTA blood samples were collected from patients who were seen at the Colombo South Teaching Hospital (CSTH) for routine reticulocyte count. Automated reticulocyte count was obtained from a Mindry BC-6800 automated analyzer and the manual reticulocyte count was performed using new Methylene blue as the supra-vital stain. Statistical analysis was done by correlation analysis and paired sample t-test using SPSS software.

Results: The mean value of the automated reticulocyte count is 3.3724% and the manual reticulocyte count is 3.3500%. There is a strong positive correlation between the automated reading and the manual reading with r= 0.982 and p=0.01. According to the results of the paired sample t-test, there is no significant difference between the automated reading and the manual reading (at a 5% level of significance).

Conclusion: There is no significant variance between automated and manual reticulocyte count. Therefore, both methods apply to performing reticulocyte counts depending on laboratory facilities and necessity. This finding is useful as many resource-poor laboratories still depend on the manual count in many peripheral areas of the country and worldwide.

Key Words: Reticulocyte count, Automated method, Manual method

1. Introduction

Reticulocyte count is a blood test that measures erythropoietic activity(1) which is the bone marrow's ability to produce red blood cells. Reticulocytes are produced and released into the blood and if the reticulocyte count is too high or too low, it indicates a serious health problem including anaemia and disorders of the bone marrow, liver, and kidney(10). It is highly used for the diagnostic purposes(2) such as various causes of anaemia, to monitor bone marrow response and the returns of bone marrow function following chemotherapy, bone marrow transplant, and post-treatment follow-up for iron deficiency anaemia, vitamin B12/Folate deficiency anaemia, or renal failure(3).

Nowadays, estimation of reticulocyte count is commonly performed with an automated analyzer, however, as many rural and under-resourced laboratories in many countries still do it manually, it is important to determine if there is a significant difference between the two methods. The manual reticulocyte counting by microscopy is the traditional method and has been considered the standard since 1940, for its simplicity and low cost(4). However, the observer's visual activity, patience, and the technician's experience to distinguish reticulated cells from other cells with inclusions that are also stained with the dye, besides the quality and

the resolution power of the microscope have been identified as other important factors that affect the accuracy of the manual reticulocyte count(5).

A previous research study by Ali and Moiz evaluated the manual method as a reliable method for under-resourced countries. Visual reticulocyte enumeration was evaluated for comparability, within-batch precision, and cost. Visual counting of reticulocytes can be used as a reliable tool for estimating reticulocytes in resource strained countries as it is not only cost-effective but can also efficiently discriminate between high and low reticulocyte ranges which are required for sound clinical judgment(13). In two other studies conducted on reticulocyte count and its parameters comparing automated analyzers, flow cytometry, and manual method, they concluded that there is a strong correlation between manual and automated methods as well as among the automated analyzers(10)(11). Even though there was no significant variance in both methods, the reference range of reticulocyte values was dependent on the method used(11) and researchers preferred the automated method as it is rapid, easy to operate, and can count a high number of cells with precise measurement(10). Trupti *et al* suggested that automated count is preferable compared to the manual method, as it is less time-consuming, highly precise, and is required for certain diseases where reticulocyte parameters are necessary(12). A study aimed to compare manual and automated methodologies for reticulocyte counting and evaluate random and systematic errors and the results obtained showed that the difference between the two methods was very small, 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 adequate clinical use(4). However, Aravinder *et al*(2016) concluded that the automated method was found to be a more reliable, dependable, and excellent method for estimating reticulocyte count than its peer methods(14).

In Sri Lanka, reticulocyte count estimation is performed by manual as well as automated methods depending on the laboratory facilities and its budget. A report of reticulocyte count issued by a laboratory should be precise, reliable, and accurate regardless of the method of analysis. Therefore, this research study was designed to determine whether there is any significant variance between manual and automated methods of reticulocyte count.

2. Methodology

This is an analytical cross-sectional study with laboratory investigations. After obtaining the ethical review committee approval from the University of Sri Jayewardenapura, Sri Lanka, the study was commenced. Specimen collection and analytical study were carried out at the Heamatology unit laboratory of CSTH. Blood specimens (EDTA anti-coagulated) were obtained by the laboratory for routine estimation of reticulocyte count from patients who attended the CSTH. The sample size was automatically calculated by Minitab version 16 for the paired-sample t-test and gave the result of 44 specimens.

The blood specimen was collected appropriately in an EDTA anti-coagulated container and received according to the protocol from both male and female patients, attending CSTH for routine estimation of reticulocyte count. Blood samples that were not either collected properly/ clotted/ haemolysed / inappropriately labelled or contained inadequate volume, were excluded.
Each sample was processed in two methods separately to estimate the reticulocyte count.

2.1 Automated Method

Initially, blood specimens were selected according to the inclusion and exclusion criteria. Then a code number was assigned to each sample and labelled. The blood sample was processed in the automated analyzer “BC-6800 MINDARY” and the reticulocyte count was obtained as a report along with the Full blood count (FBC).

2.1.1 The principle of automated reticulocyte count

The general principle of automated blood cell analysis can be discussed under three methods.

1. Electronic impedance
2. Radiofrequency

3. Optical scatter

The principle of the automated analyzer varies from the analyzer to analyzer. In this study, BC-6800 MINDRY is used to calculate automated reticulocyte count which works with modified optical light scatter method modified and called SF cube technology.

SF Cube is a path-breaking technology for reliable blood cell analysis, including WBC differential, Reticulocytes, and NRBC with efficient flagging. After reaction with proprietary reagents, the targeted blood cells undergo 3D analysis using information from the scattering of laser light at two angles and fluorescence signals. The 3D scattergram builds the power to better identify and differentiate blood cell populations, especially to reveal abnormal cell populations undetected by other techniques. Red blood cell analysis with SF cube technology helps differentiation of reticulocytes from mature red cells by their reaction with fluorescent stain(9).

2.2 Manual Method

Initially freshly prepared 1% new methylene blue was filtered into a glass bottle by using a funnel, a glass rod, and a filter paper before preparing for manual counting. Then selected blood specimens were labelled. One drop of filtered new methylene blue was added into a clean labelled Khan tube using a clean Pasteur pipette, followed by adding two drops of well-mixed EDTA anti-coagulated blood and mixed well. Khan tube was sealed with cotton wool and kept in the incubator for 20 minutes at 37^0C. After 20 minutes, the tube was mixed well and a drop of stained blood was transferred to an oil-free clean labelled glass slide, by using a clean Pasteur pipette. Thin blood film was made with the help of a clean spreader and the slide was allowed to air dry. After that, the slide was focused initially at low power and screened to check the quality of the stain. Then the region was selected where the red blood cells were separately visible without overlapping. Reticulocytes were counted just before the tail part under the oil immersion objective of a light microscope in 10 different field. Reticulocyte percentage was calculated. During the procedure, all measures were taken to avoid contamination and cross-contamination. The manual reticulocyte count was performed according to the protocol in the Dacie and Lewis practical haematology, 7th edition. It is assumed that 100 RBCs appear in one field therefore, the number of reticulocytes in 10 fields under the oil immersion objective of the microscope was counted. Finally, the reticulocyte count percentage was calculated.

$$Reticulocyte\ Percentage = \frac{No.of\ Reticulocytes}{Total\ number\ of\ RBC} \times 100$$

2.2.1 The principal of the manual method of reticulocyte count estimation

Reticulocytes contain ribosomal RNA in the cytoplasm. Ribosomes have a specific property of reacting with certain basic dyes to form a bluish-purple or violet-blue precipitate of granules(6). When unfixed reticulocytes are stained with a supra-vital stain such as new methylene blue or brilliant cresyl blue, ribosomal RNA is precipitated and stained to appear in form of a reticular network and as the cells are still alive when exposed to the dye, this is referred as supra-vital staining. An isotonic solution of supra-vital stains such as New Methylene Blue or Brilliant cresyl Blue is incubated with a few drops of blood. The red cells are stained while they are still alive (Not fixed). A thin film is made and then the reticulocytes are counted microscopically. 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 intensity/type of staining determines 4 types of reticulocyte groups. The amount of reticulum in the reticulocyte varies from a large clump in the most immature cells (Group 1 reticulocytes) to fewer granules in the most mature forms(Group IV)(4).

Technological innovations have been making reticulocyte count results more and more reproducible and exact for clinical use when compared with the manual method(7). Despite the great advances and advantages that reticulocyte count automation may offer, commercial kits for the method are highly expensive and are not widely used in clinical laboratories which do not have the technology.

3. Results

Statistical analysis of the results between manual and automated methods was tested using SPSS statistical software.In this study, 63 blood samples of patients were analyzed who came to the CSTH for reticulocyte count.

3.1 Descriptive statistics of the results among the study population

Among 63 samples minimum values obtained in automated and manual reticulocyte count are 0.37% and 0.10% and the maximum value obtained for automated and manual reticulocyte count are 36.62% and 34.00%, respectively. The mean value of the automated reticulocyte count is 3.3724% and the manual reticulocyte count is 3.3500%.

Table 01 - Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Automated	63	.37	36.62	3.3724	5.58980
Manual	63	.10	34.00	3.3500	5.37943
Valid N (list wise)	63				

In automated reticulocyte count among the 65 samples, 43 reticulocyte count results are in the reference range which is 66.2% and 20 reticulocyte count results are above the reference range which is 30.8%. Two of the reticulocyte count results are considered outliers and excluded from the analysis among the 65 samples.

Table 02 - Frequency Table of the results among the study population- Automated Code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	In the reference	43	66.2	68.3	68.3
	Above the reference	20	30.8	31.7	100.0
	Total	63	96.9	100.0	
Missing	System	2	3.1		
Total		65	100.0		

Manual reticulocyte count among 65 samples, 6 reticulocyte count results are below the reference range that is 9.2% and 34 reticulocyte count results are is in the reference range that is 52.3%. Moreover, 23 reticulocyte count results are above the reference range which is 35.4%. Two of the reticulocyte count results are considered outliers among the 65 samples and excluded from the analysis.

Table 3

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Below the reference	6	9.2	9.5	9.5
	In the reference	34	52.3	54.0	63.5
	Above the reference	23	35.4	36.5	100.0
	Total	63	96.9	100.0	
Missing	System	2	3.1		
Total		65	100.0		

Table 04 - Correlation Coefficient of the results among the study population

		Automated	Manual
Automated	Pearson Correlation	1	.982**
	Sig. (2-tailed)		.000
	N	63	63
Manual	Pearson Correlation	.982**	1
	Sig. (2-tailed)	.000	
	N	63	63
**. Correlation is significant at the 0.01 level (2-tailed).			

3.2 Analysis of Correlation Coefficient of the results among the study population

The Pearson correlation coefficient of reticulocyte count results of 63 samples was calculated. There is a strong positive correlation between automated reading and manual reading, r= 0.982, p=0.01.

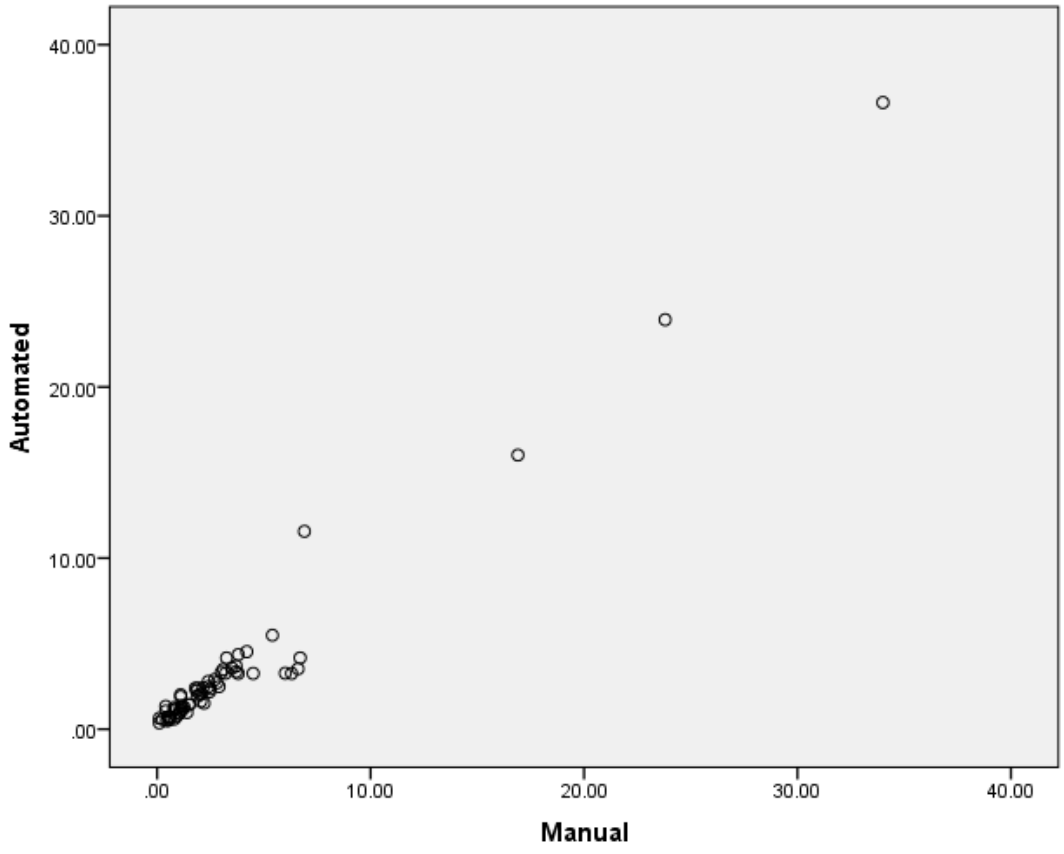

Figure 01: Correlation between manual and automated reticulocyte count

3.3 Paired sample statistics of the results among the study population

A paired sample t-test was conducted to compare automated and manual reticulocyte count. There was no significant variance in the scores for automated (M=3.3724, SD= 5.58980) and manual reticulocyte count (M=3.3500, SD=5.37943) conditions; t(62)=0.166 p=0.869.

Table 05 -Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Automated	3.3724	63	5.58980	.70425
	Manual	3.3500	63	5.37943	.67774

Table 06-Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Automated & Manual	63	.982	.000

Table 07 - Paired Samples Test

			Pair 1
			Automated - Manual
Paired Differences	Mean		.02238
	Std. Deviation		1.07301
	Std. Error Mean		.13519
	95% Confidence Interval of the Difference	Lower	-.24785
		Upper	.29261
T			.166
Df			62
Sig. (2-tailed)			.869

Therefore, no significant difference was found between the automated reading and the manual reading at the 5% level of significance.

4. Discussion

This analytical cross-sectional study was carried out to compare the results of automated and manual reticulocyte count. In this study, it is concluded that there is no significant variance between the results of manual and automated reticulocyte count supported by previous studies(12). Therefore both method provides reliable results for adequate clinical use when well-conducted(15).

The minimum sample size calculated for the study is 44. The samples analyzed in this study were 63, excluding 2 outliers among the 65 samples that were tested. Therefore, the higher sample size made the statistical validity of the study better.

Several previous studies conducted to compare automated and manual reticulocyte count stated that the manual method of reticulocyte count leads to erroneous results(10). The causative factors for the erroneous results were the consummation of more time, lack of staining quality, calculating a low number of cells, inter-observer variation, lack of reproducibility, inappropriate techniques, inappropriate counting and

calculation, inappropriate blood films, higher chance of contamination and cross-contamination. In this study, all measures were taken care of to minimize these errors.

Several precautions were taken to minimize the time consumed for a manual count. Samples were processed within 6-8 hours because reticulocyte count tends to decrease with time unless it was kept at 4℃. All samples were performed as soon as possible after receipt to the laboratory of CSTH Samples preparation, incubation, and slide preparation was done as soon as possible without delay.

The staining quality of the manual method was improved in several ways to maintain the same staining quality of dye used in the automated method. Staining quality was enhanced by using freshly prepared New methylene blue. As staining particles present in the stain can contaminate blood films, deposit on the RBC and appear as reticulocytes leading to misidentification, the stain was filtered daily before use with a clean funnel and glass rod. Samples were incubated in a water bath at 37℃ for 20 minutes to enhance the quality of staining.

An automated analyzer counts a higher number of cells using the optical scattering technique which is known as SF cube technology(16). The manual method counts reticulocytes among 1000 RBCs which is very less in number compared to the automated method. Even though the number of cells counted in the manual method is less, it is adequate to provide reliable results.

Inter-observer variation which can happen in the manual method was avoided by subjecting one person to observe all the prepared slides throughout the process. All labelled slides were properly stored in the slide box according to their prepared date and sample number to enhance their reproducibility. The investigator practiced two days before the study commenced to improve the technical aspects of performing the manual method and to enhance practical knowledge of distinguishing reticulocytes from stained red blood cells. Several precautions were taken to minimize and avoid contamination and sample cross-contamination.

5. Conclusion

It is widely accepted that the automated reticulocyte count is effective, fast, and able to analyze a higher number of samples in one go while the manual method consumes more time and the chances of error are high compared to the automated method. However, this study concludes that both methods provide accurate results without significant variation when blood samples are handled appropriately and the standard operating procedures are strictly followed and performed by skilled laboratory personnel. This study highlights that it is important to practice the manual method fulfilling the standard operation procedures, especially in the under-resourced laboratories if the automated method is not accessible due to various reasons. This is paramount in importance as quality healthcare has to be provided with proper and accurate laboratory results when needed, even in the peripheral/under-resourced health sectors in a country.

Limitations and Recommendations

In this study, the automated results rely on the Mindry BC- 6800 automated analyzer in the main laboratory of CSTH. The manual method was carried out by one laboratory personnel so the results are highly dependent on the skills of that laboratory personnel.

Future studies should be developed considering various automated analyzers and manual methods using different stains and procedures. The above study can be modified further by analyzing blood samples separately from males and females. Moreover, blood samples can be collected from healthy individuals other than from patients with haematologic disorders as well. The study can be improved by considering each disease condition separately where reticulocyte count is requested and increasing the sample size also will lead to statistically more significant results.

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Conflict of Interest

None declared.

References

1. D'Onofrio G, Kim YR, Schulze S, Lorentz T, Dörner K, Goossens W, et al. Evaluation of the Abbott Cell Dyn 4000 automated fluorescent reticulocyte measurements: Comparison with manual, FACScan and Sysmex R1000 methods. Clin Lab Haematol. 1997;19(4):253–60.
2. Nobes PR, Carter AB. Reticulocyte counting using flow cytometry. J Clin Pathol. 1990;43(8):675–678.
3. Kageoka T. Reticulocyte as indication of the erythroid hematopoiesis: reticulocyte fractions in peripheral blood and bone marrow. Rinsho Byori. 2001;49(5):485-489
4. Simionatto M, De Paula JP, Chaves MAF, Bortoloso M, Cicchetti D, Leonart MSS, et al. Manual and automated reticulocyte counts. Hematology. 2013;15(6):406–409.
5. Pappas AA, Owens RB, Flick JT. Reticulocyte counting by flow cytometry: A comparison with manual methods. Ann Clin Lab Sci. 1992;22(2):125–32.
6. Riley RS, Ben-Ezra JM, Goel R, Tidwell A. Reticulocytes and reticulocyte enumeration. J Clin Lab Anal. 2001;15(5):267–94.
7. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: State of the art and clinical applications in the evaluation of erythropoiesis. Clinical Chemistry and Laboratory Medicine. 2010;48(10):1369-1380
8. SEED Haematology – The importance of reticulocyte detection; Sysmex Educational Enhancement and Development | January 2016
9. Urrechaga E, Bilbao S, Merino M. Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800+ counter in the study of erythropoiesis. Clinica Chimica Acta. 2019 Jun 1;493:S427
10. Patel K, Patel SM. Comparison of automated flowcytometric reticulocyte analysis with manual reticulocyte count. Int J Res Med Sci. 2019;7(10):3825.
11. Uppal V, Naseem S, Bihana I, Sachdeva MUS, Varma N. Reticulocyte count and its parameters: comparison of automated analyzers, flow cytometry, and manual method. J Hematop. 2020;13(2):89–96.
12. Gorte TR, Deshmukh AV, Gangane NM. Automated versus manual method for reticulocyte count : A comparative study in rural central India. 2020;145–9.
13. Ali AF, Moiz B, Omer S. Is manual reticulocyte count a reliable option for under resourced countries? J Pak Med Assoc. 2010;60(11):892–6.
14. Singh A, Rastogi S, Garg D. Automated Reticulocyte Count Wins Over Manual Methods Authors. J Med Sci Clin Res. 2016;04(04):10231–3.
15. Simionatto M, Paula JP De, Ana M, Chaves F, Bortoloso M, Cicchetti D, et al. Manual and automated reticulocyte counts Manual and automated reticulocyte counts. 2013;8454.
16. Buttarello M, Rauli A, Mezzapelle G. Reticulocyte count and extended reticulocyte parameters by Mindray BC-6800: Reference intervals and comparison with Sysmex XE-5000. Int J Lab Hematol. 2017;39(6):596–603.