Open Access

Original Article

Comparative Analysis of Manual and Automated Reticulocyte Count in Patients Referred for Haemoglobin Studies in A Tertiary Care Setting: A Cross-Sectional Study

Saad Yousof, Manzar Bozdar, Mohib Shamoon, Rafia Mahmood, Ayesha Khurshid, Aysha Khan, Saqib Hussain Korejo, Syeda Samia Shafaat

Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To compare the effectiveness and accuracy between manual and automated reticulocyte counting methods, and to assess the agreement between these methods.

Study Design: Analytical ross-sectional study.

Place and Duration of Study: Department of Hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from Jul 2024 to Feb 2025.

Methodology: Reticulocyte counts were simultaneously determined by manual microscopic counting and automated counting using the Sysmex XN-3000 hematology analyzer. Comparability, intra-batch precision, correlation, and cost-effectiveness of both methods were evaluated. Data analysis included descriptive statistics, Spearman's correlation, and Bland-Altman agreement analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: A total of 189 patient samples were analyzed, comprising 108(57.1%) males and 81(42.9%) females, with a median age of 20.0 years. Manual reticulocyte counts had a median of 1.80% (Range: 0.2–10.5%), whereas automated counts showed a median of 2.20% (Range: 0.3–12.6%). Spearman's rank correlation demonstrated a strong positive correlation between the two methods (ρ=0.960, p<0.001). Bland-Altman analysis revealed a mean bias of 0.44% (95% Confidence Interval: 0.357–0.531%), indicating slight systematic overestimation by the automated method. Using manual criteria, 102(54.0%) samples were classified as normal (1.0–2.5%), whereas the automated method similarly classified 90(47.6%) samples. The automated method categorized more samples as high (>2.5%) compared to manual counting (40.2% vs. 28.0%).

Conclusion: Microscopic manual reticulocyte counting is a reliable method for evaluating reticulocyte counts in resource-constrained areas as it possesses the ability to effectively distinguish between high and low reticulocytes levels crucial for clinical decision-making.

Keywords: Automation, Clinical Laboratory Techniques, Hematology, Reticulocyte Count.

How to Cite This Article: *Yousof S, Bozdar M, Shamoon M, Mahmood R, Khurshid A, Khan A, Korejo SH, Shafaat SS. Comparative Analysis of Manual and Automated Reticulocyte Count in Patients Referred for Haemoglobin Studies in A Tertiary Care Setting: A Cross-Sectional Study. Pak Armed Forces Med J 2026; 76(Suppl-1): S173-S177. DOI: https://doi.org/10.51253/pafmj.v76iSUPPL-1.13338*

INTRODUCTION

Reticulocytes are immature red blood cells that contain intracellular ribonucleic acid (RNA) and ribosomes,[1] with increased reticulocytes number in blood reflecting active bone marrow while reduction is indicative of hypoactivity,[2] serving as a key indicator of red cell production activity.[3] Hence, an elevated reticulocyte count reflects acute blood loss, haemolysis, or recovery following treatment for iron, vitamin B12, or folate deficiencies.[4] Conversely, uncorrected nutritional deficiencies or marrow failure typically result in low reticulocyte counts. Monitoring reticulocyte levels offers a practical approach to assess the efficacy of erythropoietin therapy and can also be used to predict the recovery from chemotherapy or transplanted bone marrow in patients with aplastic anemia or cancer.[2,5] Currently, two primary methods are employed to determine reticulocyte count, one being the traditionally used manual method and the other bring an automated method.[5] which involves labelling reticulocytes with polymethine or similar fluorescent dyes for automated measurement based on fluorescence intensity, providing additional indices such as the immature reticulocyte fraction (IRF), mean reticulocyte cell volume, and reticulocyte haemoglobin (Ret Hb) content,[6] but despite its accuracy, factors such as cost of equipment and reagent availability remain an obstacle. Another approach to reticulocyte counting is through flow cytometry and is reported to have significant advantages over manual counts,[7,8] but presence of nucleated red cells, Howell–Jolly bodies, sickle cells, or giant platelets may interfere with the precision of reticulocyte counts.[8] Therefore, manual counting by light microscopy with supravital dyes for

Correspondence: Dr Saad Yousof, Department of Hematology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

Received: 27 Mar 2025; revision received: 11 Jul 2025; accepted: 12 Jul 2025

RNA, remains the standard,[9] with commonly used supravital dyes being brilliant cresyl blue and new methylene blue.[10] The aim of this study was to determine the correlation between manual and automated methods of reticulocyte counting in patients as manual reticulocyte count is a cheap and inexpensive method as compared to automated method.

METHODOLOGY

This was an analytical cross-sectional study which was conducted in the Department of Haematology, at Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. Ethics approval was obtained from Institutional Review Committee (ERC Number: FC-HEM23-20/READ-IRB/24/3556) and the study spanned eight months, commencing in July 2024 and concluding in February 2025. The sample size for this study was calculated using G*Power software (version 3.1.9.7), employing a paired-sample t-test (two tailed) to compare manual and automated reticulocyte count percentages obtained from the same patient samples where an effect size (dz) of 0.237, representing the mean difference in reticulocyte percentages between manual and automated methods, was derived from previously published literature.[11] With a desired statistical power of 90% and keeping a significance level (α) at 0.05, a total sample size of 189 was calculated, attained using non-probability consecutive sampling. Blood samples approximately 3ml in volume were collected from each participant, after taking informed, written consent and processed within 4 hours of collection.

Inclusion Criteria: Patients of all ages, from both genders, referred for haemoglobin studies were included.

Exclusion Criteria: Samples received from outside the hospital, those with insufficient blood volume and anticoagulant other than Ethylenediaminetetraacetic acid (EDTA) were not included.

Manual reticulocyte counting was done using conventional method where one drop of blood from EDTA vial and one drop of retic stain (supravital dye) were taken in test tube and incubated for 20 minutes in water bath at 37°C. Smear was prepared on glass slide and allowed to air dry. The slide was than examined under microscope (CX23 Olympus, Japan) at 100x magnification by using the oil immersion lens for identification and counting of reticulocytes. One thousand red cells were counted on each smear and the percentage of cells containing stained RNA was calculated microscopically by two different qualified observer. To reduce observer-related variability, rigorous steps were taken as per previously validated approaches. Both observers underwent standardized training, each blood smear was independently examined by both, who were blinded to each other's results, and ocular insets were not utilized to avoid counting bias. The microscope was calibrated for optimal focus and light intensity to ensure clear differentiation of reticulocytes. The final reticulocyte count was determined by calculating the mean of these independent assessments, a procedure supported by prior studies.[11] For the automated reticulocyte count the same samples were run using a Sysmex XN-3000 hematology analyzer, using its reticulocyte mode following the manufacturer's protocols. The RNA residues in reticulocytes were marked with fluorescent dye polymethine which penetrated the cell membrane and stained them. Reticulocyte quantification was based on the forward light scatter principle and reticulocytes were classified into four categories according to fluorescence intensity: High Fluorescence Ratio (HFR), Medium Fluorescence Ratio (MFR), Low Fluorescence Ratio (LFR) and Immature Fluorescence Ratio (IFR). The instrument also provided additional parameters such as Immature Reticulocyte Fraction (IRF), which is combined percentage of MRF and HFR indicating the proportion of immature reticulocytes in circulation, and the reticulocyte hemoglobin content (Ret-Hb), representing the hemoglobin content of reticulocytes. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. The normality of quantitative variables (age, manual reticulocyte count, and automated reticulocyte count) was assessed by the Shapiro-Wilk test, and all variables were found to be non-normally distributed, hence, they were presented as median and interquartile range (IQR). Qualitative variables (gender and reticulocyte count categories of low, normal, high) were presented as frequencies and percentages. Wilcoxon signed-rank test was applied to compare manual and automated reticulocyte counts and correlation between manual and automated reticulocyte counts was analyzed using Spearman's rank correlation coefficient. Agreement analysis between the two methods was evaluated using Bland-Altman plots and a *p*-value of ≤0.05 was considered statistically significant. The coefficient of variation for manual counting was 18.5%, compared to 8.7% for the automated method, highlighting the superior precision of the automated analyzer. Cost analysis

revealed that manual counting was significantly less expensive compared to automated counting which cost four times more than manual counting.

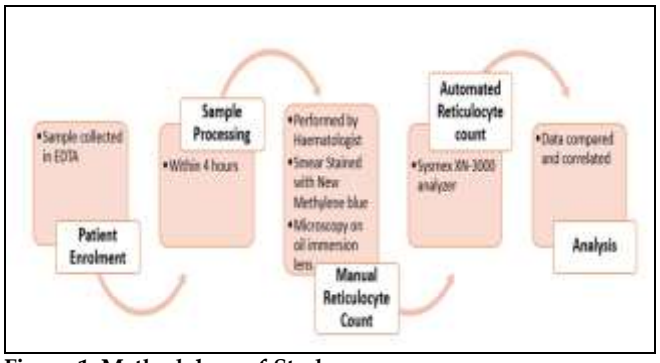

Figure-1: Methodology of Study

RESULTS

A total of 189 patient samples were analyzed, comprising 108(57.1%) males and 81(42.9%) females. The median age of patients was 20.0 years (IQR: 12.0–30.0 years), ranging from 1 to 50 years. Hypochromic microcytic anaemia was the most common clinical indication for reticulocyte counting. Descriptive statistics for reticulocyte counts showed manual counts ranging from 0.2% to 10.5%, with a median of 1.80% (IQR: 1.20–2.70%), and automated counts ranging from 0.3% to 12.6%, with a median of 2.20% (IQR: 1.57–3.10%). Correlation between manual and automated reticulocyte counts was assessed using Spearman's rank correlation coefficient, revealing a statistically significant positive correlation (ρ=0.960, p<0.001). This indicated consistent associations between higher counts measured by one method and higher counts measured by the other, where the red dashed line in the correlation plot represents the line of identity, illustrating agreement between methodologies as shown in Figure-2.

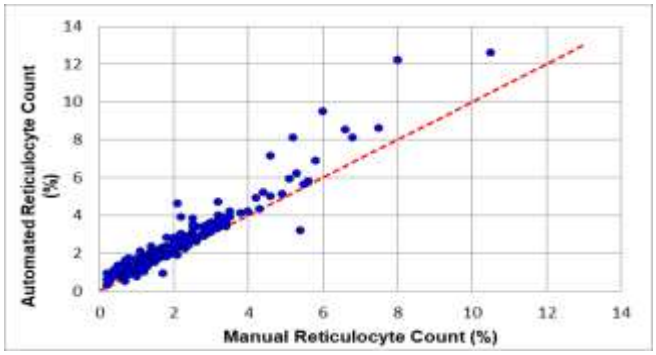

Figure-2: Correlation Between Manual and Automated Reticulocyte Count (n=189, Spearman's Rank Correlation Coefficient (ρ) Used to Assess Correlation, Red Dashed Line Represents Line of Identity)

Agreement between the two methods was further analyzed using Bland-Altman plotting where mean bias of 0.44% (95% CI: 0.357%–0.531%) was observed, indicating systematic slight overestimation by the automated method compared to the manual method as seen in Figure-3.

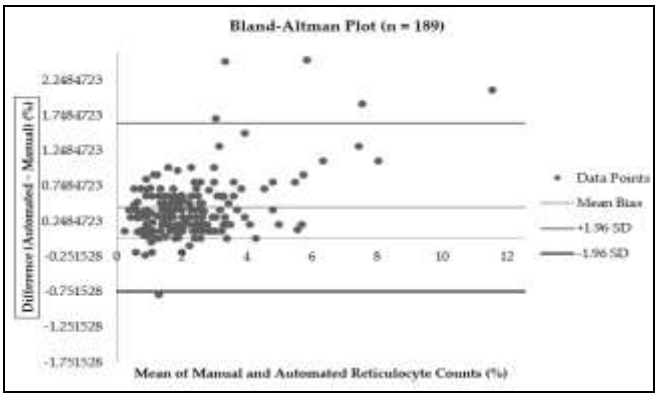

Figure-3: Bland-Altman Plot Illustrating Agreement Between Manual and Automated Reticulocyte Counts (n=189)

Using the manual method, 34(18.0%) samples had low reticulocyte counts (<1%), 102(54.0%) were within the normal range (1–2.5%), and 53(28.0%) had high counts (>2.5%). Using the automated method, 23(12.2%) samples were classified as low, 90(47.6%) as normal, and 76(40.2%) as high, as summarized in Table-I.

Table-I: Distribution of Reticulocyte Count Values (n=189)

Parameters	Manual Method (n=189)	Automated Method (n=189)
Median (IQR) (%)	1.80 (1.20 - 2.70)	2.20 (1.57 - 3.10)
Range (%)	0.20 - 10.50	0.30 - 12.60
Distribution by clinical ranges, n (%):		
Low (<1%)	34 (18.0%)	23 (12.2%)
Medium (1-2.5%)	102 (54.0%)	90 (47.6%)
High (>2.5%)	53 (28.0%)	76 (40.2%)

IQR: Interquartile Range

Table-II: Comparison of Manual and Automated Reticulocyte Counting (n=189)

Method	Median (IQR)	*p*-value
Manual Reticulocyte Count	1.80 (1.20–2.70)	<0.001
Automated Reticulocyte Count	2.20 (1.57–3.10)	

IQR: Interquartile Range

There was a statistically significant difference between manual and automated reticulocyte counts, with the automated method generally reporting higher values and demonstrating a wider variability. As shown in Table II, this reflects a tendency of the automated system to classify more samples at the

higher end of the measurement range compared to manual microscopy, highlighting the consistent upward shift observed between the two methods. Automated reticulocyte counting yielded consistently higher reticulocyte values and demonstrated greater variability compared to manual counting (p<0.001).

DISCUSSION

Reticulocyte counting plays an important role in diagnosing and monitoring various haematological disorders and their accurate assessment is essential to clinical decision-making, guiding therapy, and evaluating patient response to treatment. In our study we compared the manual and automated reticulocyte counting methods, and it showed strong correlation (Spearman's rho=0.960, p<0.001), which were aligned with previous published data highlighting similar strong correlations between these two methods in clinical hematology practice,[12] while the results of another study showed strong correlation of reticulocyte estimation obtained by manual and automated method (r value of 0.884).[2,11] The Bland-Altman analysis revealed a slight bias of 0.44% with 95% limits of agreement ranging from -0.75% to 1.64%, indicating that automated counting consistently produced slightly higher reticulocyte values than the manual method with similar findings reported in literature, attributed primarily to the higher sensitivity of automated analyzers to detect immature reticulocytes.[13] Our study involved a heterogenous population, from regions known for higher prevalence of nutritional and genetic anaemia.[14] In our study, we identified discrepancies between manual and automated reticulocyte counting methods where 12.2% of the samples classified as "normal" using the manual method were categorized as "high" by the automated method while 6.9% of samples categorized as "normal" by automated analysis were classified as "low" (<1%) when evaluated manually which is somewhat higher compared to international findings, where discrepancies generally ranged from 3% to 8%.[15,16] These observed differences, particularly the upward shift from manual to automated counts, could carry clinical implications by influencing patient management decisions, especially in borderline cases. Previous studies support our observations, highlighting manual counting's tendency toward variability and potential inaccuracies compared to the precision and consistency offered by automated analyzers.[13,17] Moreover, automation reduces human-related errors inherent in manual counting techniques, emphasizing its advantage for routine clinical practice, despite higher associated costs.[18] With the rising volume of laboratory samples and the growing need for detailed study of complex diseases, automated hematology analyzers have become increasingly accurate through continuous technological advancements.[19,20]

LIMITATIONS OF STUDY

The single-center nature of the study may constrain the extent to which the findings can be generalized. Furthermore, manual counting was performed by hematologists, which might not reflect variability in less controlled settings.

CONCLUSION

This study demonstrated that automated reticulocyte counting provides superior accuracy and precision compared to manual methods, making it a viable, cost-effective option for resource-limited settings or routine screening, though its variability necessitates rigorous quality control near clinical decision thresholds, so laboratories should ultimately select the method best aligned with their budgetary constraints, staff capabilities, and patient care requirements.

ACKNOWLEDGEMENT

We are grateful to everyone who assisted, whether directly or indirectly, in the execution of this research.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SY & MB: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

MS & RM & AK: Data acquisition, data analysis, approval of the final version to be published.

AK & SHK & SSS: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. George L, Basu D, Kar R. Comparison between manual and automated methods of counting reticulocytes and the effect of sample storage on reticulocyte count: a cross-sectional study from Southern India. Indian J Hematol Blood Transfus 2022; 38(1): 106-110. https://doi.org/10.1007/s12288-021-01424-x
2. Baker RM, Fernando MG, Fernando KM. Comparison of automated and manual reticulocyte count in a cohort of patients' samples in Haematology Laboratory of Colombo South Teaching Hospital, Sri Lanka. Emerg Med Sci 2022; 6(2): 23-30.

3. Kumari R, Chatterjee RC. Comparative assessment of manual and automated methods of counting reticulocytes and the effect of sample storage on reticulocyte count: an analytical assessment. Int J Pharm Clin Res 2022; 14(10): 592-598.
4. Stevens-Hernandez CJ, Flatt JF, Kupzig S, Bruce LJ. Reticulocyte maturation and variant red blood cells. Front Physiol 2022; 13: 834463. https://doi.org/10.3389/fphys.2022.834463
5. Singh M, Mishra VP. Manual versus automated method for reticulocyte count: a comparative study. IP J Diagn Pathol Oncol 2024; 9(1): 44-48. https://doi.org/10.18231/j.jdpo.2024.008
6. Lin H, Zeng B, Shi X, et al. The mean reticulocyte volume is a valuable index in early diagnosis of cancer-related anemia. PeerJ 2024; 12: e17063. https://doi.org/10.7717/peerj.17063
7. Yahagi K, Arai T, Katagiri H, et al. Performance evaluation of a novel reticulocyte identification method using metachromatic nucleic acid staining based on a crossover analysis of emission DNA/RNA light (RNP Determination™) in hematology analyzer Celltac G+. Int J Lab Hematol 2022; 44(6): 1050-1059. https://doi.org/10.1111/ijlh.13947
8. Pappas AA, Owens RB, Flick JT. Reticulocyte counting by flow cytometry: a comparison with manual methods. Ann Clin Lab Sci 1992; 22(2): 125-132.
9. Riley RS, Ben-Ezra JM, Goel R, Tidwell A. Reticulocytes and reticulocyte enumeration. J Clin Lab Anal 2001; 15(5): 267-294. https://doi.org/10.1002/jcla.1039
10. Pan LL, Yu HC, Lee CH, et al. Impact of staining methods and human factors on accuracy of manual reticulocyte enumeration. Diagnostics (Basel) 2022; 12(9): 2154. https://doi.org/10.3390/diagnostics12092154
11. Farzand Ali A, Bilwani M, Omer S. Is manual reticulocyte count a reliable option for under-resourced countries? J Pak Med Assoc 2010; 60(11): 892-896.
12. Buttarello M, Plebani M. Automated blood cell counts: state of the art. Am J Clin Pathol 2008; 130(1): 104-116. https://doi.org/10.1309/EK3C7CTDKNVPXVTN
13. Brugnara C, Mohandas N. Red cell indices in classification and treatment of anemias: from M.M. Wintrobe's original 1934 classification to the third millennium. Curr Opin Hematol 2013; 20(3): 222-230. https://doi.org/10.1097/MOH.0b013e32835f5933
14. Ansari SH, Shamsi TS, Ashraf M, et al. Molecular epidemiology of β-thalassemia in Pakistan: far reaching implications. Int J Mol Epidemiol Genet 2011; 2(4): 403-408.
15. Agarwal AM, Rets A. Laboratory approach to investigation of anemia in pregnancy. Int J Lab Hematol 2021; 43(Suppl 1): 65-70. https://doi.org/10.1111/ijlh.13551
16. Sun S, Wang G, Zhang B, Wang F, Wu W. Utility of Faster R-CNN in methodological comparison and evaluation of reticulocytes. Front Physiol 2024; 15: 1373103. https://doi.org/10.3389/fphys.2024.1373103
17. 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 Hematopathol 2020; 13(2): 89-96. https://doi.org/10.1007/s12308-020-00395-8
18. Maconi M, Danise P, Cavalca L, Formisano D. Flow cytometric reticulocyte counting: a comparison between two methods. J Clin Lab Anal 2010; 24(4): 252-255. https://doi.org/10.1002/jcla.20394
19. Briggs C, Culp N, Davis B, et al. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. Int J Lab Hematol 2014; 36(6): 613-627. https://doi.org/10.1111/ijlh.12201
20. Bain BJ. Diagnosis from the blood smear. N Engl J Med 2005; 353(5): 498-507. https://doi.org/10.1056/NEJMra043442