Original Article

Comparative Evaluation of Manual and Automated Nucleated Red Blood Cell Counts in Neonates: Insights from a Rural North Indian Tertiary Care Centre

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Abstract

Background: Although a small number of nucleated red blood cells (nRBCs) in neonates is physiological, their enumeration is crucial, especially in critical care, as their presence or elevation can indicate perinatal hypoxia, fetal anaemia, or other pathologic states. Clinical laboratories often prefer automated counting due to the subjectivity and time-consuming nature of manual methods.

Methods: The primary aim was to study the comparison of nRBC counts using an automated haematologyanalyser versus the manual method. This prospective cross-sectional study was conducted from November 2024 to February 2025 with a sample size of 100 neonates aged 0–28 days. Automated counting utilised the Sysmex XN-1000 while the manual counting involved analysing a leishman-stained peripheral blood smear under a microscope, reporting nRBCs per 100 WBCs.

Result: Of the 100 cases, the majority were male (66%), and most were 0-2 days old (83%). Common symptoms included fever (56%) and jaundice (22%). Haematological analysis showed that 90% of cases had normal haemoglobin (Hb 14-24 gm%) and 86% had normal mean corpuscular volume. Based on automated counts, 40% of cases showed nRBCs less than (<1.0 %), and 39% showed 1.1-10.0 %. Manual nRBC counting showed the majority of cases (89%) were in the 0 to 10 nRBC range.

Conclusion: Both automated and manual techniques for nRBC counting yielded comparable results. Automated analysers, such as the Sysmex XN-1000, can reliably substitute for manual methods due to their accuracy, speed, and ability to reduce workload, while also helping to eliminate human error and bias.

Keywords: Analyser; Microscopy; Nucleated Red Blood Cells; Neonates

Introduction

During fetal life, erythropoiesis primarily occurs in the yolk sac (starting week three), the liver and spleen (months two and three), and finally the bone marrow (starting month five). Normoblasts, also known as erythroblasts or nucleated red blood cells (nRBCs), are immature erythrocytes that still possess a cell nucleus and are the direct progenitors of normal erythrocytes.[1] Typically, nRBCs are only found in the peripheral blood throughout fetal life and can be observed at birth and in the initial days following delivery. The presence of a small number of circulating nRBCs in neonates is physiological. Erythropoiesis is elevated in preterm neonates, leading to substantial nRBCs visible for a longer postnatal duration. In

healthy, full-term newborns, nRBCs are usually no longer found in the peripheral blood seven days after birth.[1] The typical range for nRBCs in a term infant is 0.1–0.2 x 10^9/I. However, gestational age (premature babies have greater counts), the postpartum period (counts cut in half within 12 hours of birth), and birth weight (inverse relationship) can influence nRBC counts.

Normoblastemia, defined as an increased number of nRBCs in peripheral blood compared to the typically expected level for the age group or the presence beyond approximately one week following a full-term normal birth, is considered abnormal. Elevated nRBC counts may be linked to fetal hypoxia, perinatal asphyxia, maternal diabetes, fetal anaemia, intrauterine infection, or ABO/RH blood incompatibility.[2]The presence of nRBCs has been linked to a worse prognosis in various clinical circumstances. Outside of the neonatal period, nRBCs only appear in pathological states, such as myeloproliferative disorders, regenerative anaemia, or sickle cell disease.[2]

Accurate nRBC enumeration is essential because nRBCs are frequently grouped with lymphocytes in automated haematologyanalysers, necessitating adjustment of WBC counts.[3]While the manual method (looking at a blood smear under a microscope) is subjective and time-consuming, clinical labs increasingly prefer automated haematologyanalysers for detection and quantification due to their efficiency and reliability.[4]The primary aim of our study was to study the comparison of nRBC counts using an automated haematologyanalyser versus the manual method. Objectives included studying the number of nRBC counts by microscopy and machine, and correlating these counts with peripheral smear examination.

Materials and Methods

The study was a prospective cross-sectional study undertaken in the Hematology laboratory of the M.M. Institute of Medical Science & Research (MMIMSR), Mullana Ambala, Department of Pathology. This study was conducted from November 2024 to February 2025. A total of 100 neonates were included. Neonates aged 0–28 days were included. Infants (birth to 1 year old), children, and adults were excluded, as their parents/guardians did not consent to the study. Blood was collected via venipuncture, heel puncture, or ear lobe puncture in vacutainer tubes containing EDTA (Ethylene diamine tetra acetic acid). One millilitre of EDTA venous blood was collected. Whole blood was kept for five to six hours at room temperature, or up to 24 or 48 hours in a refrigerator at 4°C.

The Sysmex XN-1000 is an automated haematologyanalyser capable of enhancing clinical parameters and performing complete blood counts (CBC), including counting RBCs, platelets, and nRBCs. The analyser uses electrical impedance, laser light scattering, and dye bonding. Specifically, it employs Flow Cytometry, Hydrodynamic Focusing, Laser Light Scattering, and Fluorescence Detection to measure cell size, complexity, and fluorescence, aligning cells for accurate measurement and quantifying cells based on fluorescent dyes. The results are displayed as a percentage of WBCs or as an absolute count. The analyser processes up to 100 CBC + DIFF samples per hour.

The manual method involves looking at a Wright-Giemsa-stained blood smear under a microscope. A thin coating of blood is applied to a glass slide, air-dried quickly, and stained with a Romanowsky-type stain, specifically Leishman stain, according to standard operating procedure. The technician examines the smear using the oil immersion lens of a microscope, conducting a standard WBC differential count and simultaneously counting the nRBCs. For each case, a total of 100 White Blood Cells (WBCs) were counted across the "monolayer" zone of the Leishman-stained smear using the oil immersion objective (100x). The number of nucleated red blood cells (nRBCs) seen during this 100-WBC count was recorded and expressed as nRBCs per 100 WBCs. This method is subjective and time-consuming.

Crucially, the comparison between the automated and manual counting methods was performed using Bland-Altman plot analysis to determine bias and limits of agreement. Data was entered using SPSS software (version 28) and Microsoft Excel.

Informed written consent from all the patients and ethical clearance (IEC-3349 dated 08.03.2023, MMIMSR, Mullana, Ambala) from the institute's ethical committee was obtained.

Results

The prospective cross-sectional study included 100 neonates and revealed a heavy concentration of cases in the youngest age groups, with 83% of neonates falling into the 0–2 days age bracket. The study population exhibited a male predominance (66%). Clinically, the most frequently reported symptoms were fever (56%) and jaundice (22%). Other symptoms included diarrhea (18%), irritability (13%), blood in urine (12%), and cough (11%).

Analysis of general hematological parameters indicated that the majority of neonates possessed normal values for key metrics, including normal hemoglobin (Hb) in 90% of cases (14-24 gm %), normal Mean Corpuscular Volume (MCV) in 86% of cases (96-108 fl), normal Total Leucocyte Count (TLC) in 90% of cases (5,000–20,000/cumm), and normal platelet counts in 88% of cases (1,50000 – 4,00000). However, MCH values were noted to be elevated, with 72% of cases showing MCH greater than (>34 pg), and PCV values were also skewed high, with 59% of cases showing PCV greater than (>48%).

For nRBC enumeration, the automated Sysmex XN-1000 method found that 79% of cases were in the lower ranges (40% were <1.0% and 39% were 1.1–10.0 nRBCs per 100 WBCs). Similarly, the manual microscopic counting method found that the majority of cases (89%) showed 0 to 10 nRBCs per 100 White Blood Cells (WBCs). (Figures-1 and 2). Table 1 and

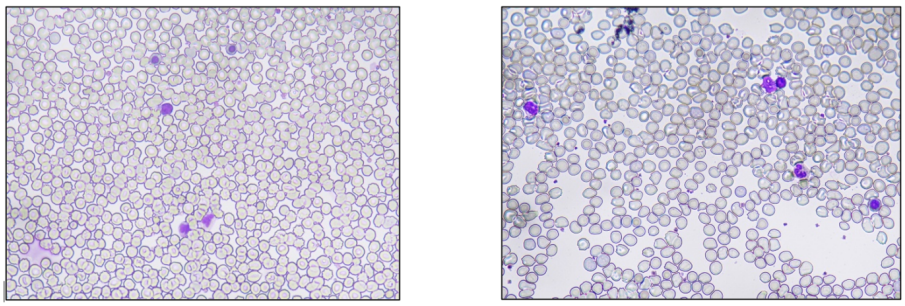

Figure 1: Peripheral blood film shows normocytic normochromic blood picture with presence of nRBCs (Leishman, x100).

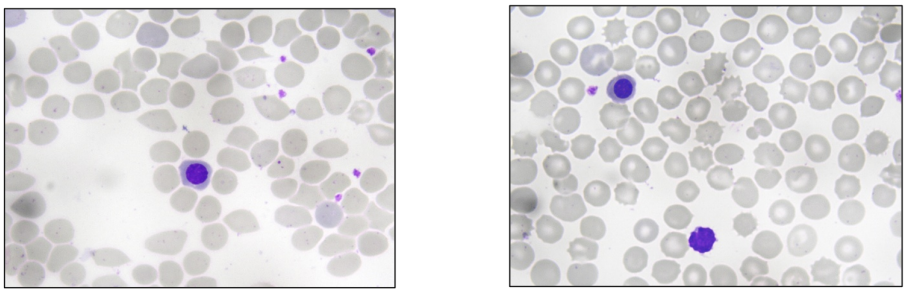

Figure 2: Peripheral blood film shows normocytic normochromic blood picture with presence of nRBCs (Leishman, x400).

Figures 3 & 4 compare nRBC counts using automated and manual methods.

Table 1: nRBC counting comparison using automated and manual methods.

Method	nRBC Range (nRBCs per 100 WBCs)	No. of cases	Percentage
Automated (Sysmex XN-1000)	<1.0 %	40	40
	1.1- 10.0 %	39	39
	10.1 – 100.0 %	19	19
	>100.0 %	2	2
Manual (Microscopy)	0 nRBCs	13	13
	1-10 nRBCs (combined)	76	76
	More than 10 nRBCs	11	11

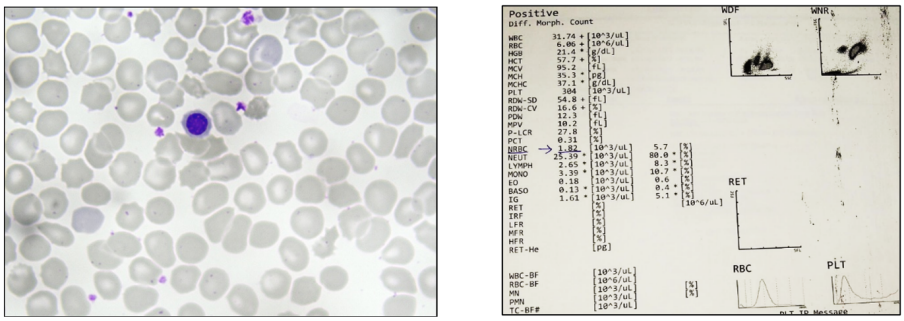

Figure 3: Peripheral blood film shows presence of nRBCs on manual counting (Leishman, x400) along with similar findings on automated report.

The Bland-Altman analysis showed

- Mean Bias: 3.55 nRBC/100 WBC. This indicates that, on average, the Sysmex XN-1000 reports slightly higher counts than the manual method.

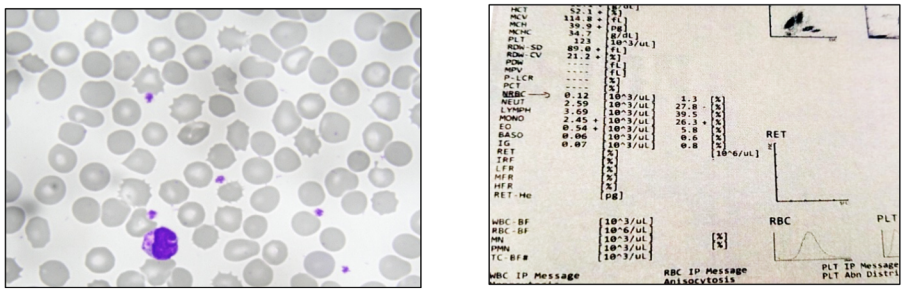

Figure 4: Peripheral blood film shows absence of nRBCs on manual counting (Leishman, x400) but detection of nRBCs is noted on automated report.

- 95% Limits of Agreement (LoA): The differences range from 21.33 to +28.43. While the majority of cases cluster near zero, the wider limits reflect the discrepancies you observed in neonates with very high nRBC counts.
- Pearson Correlation : 0.907 ($p < 0.001$), confirming a strong positive linear relationship between the two methods.

Discussion

nRBCs are an important haematological parameter in neonatal practice. They typically decrease rapidly after birth, thereby serving as indicators of erythropoietic activity and markers of perinatal stress, hypoxia and hematological disorders. Accurate quantification is essential because nRBC elevation is associated with adverse neonatal outcomes such as birth asphyxia, prematurity, maternal hypertension, and intrauterine growth restriction.[2]Traditionally, nRBC counts have been estimated manually on peripheral smear differentials; however, automated haematologyanalysers now offer nRBC quantification as part of routine complete blood counts. The present study compared manual and automated nRBC counts in neonates at a rural tertiary care centre in North India and provided insights into the reliability, agreement, and practical relevance of both approaches. Accurate quantification is essential for diagnosis, treatment decisions and prognostic evaluations.

Automated analyzers report higher sensitivity in detecting low-level nRBC, particularly in neonates with borderline counts.[5] This is consistent with previous literature pointing towards the fact that manual microscopies can underreport nRBC due to observer fatigue and smear preparation variability.[6, 7, 8] Manual examination, however, remains a critical confirming tool, especially in resource-limited settings like rural tertiary centres, where instrument flags, platelet clumping, abnormal cell morphology, or high leukocyte counts may compromise automated precision. In rural tertiary care centres, manual counts remain prevalent due to limited access to advanced automated haematology analysers. The findings in our study also support the consensus in existing literature that automated haematologyanalysers are reliable tools for nRBC enumeration. The use of automated counting can eliminate human error and bias, making the results more dependable and time-efficient.[8]

In our study, smears with poor staining quality or those prepared in emergency/ night hours showed greater discrepancies, highlighting operational challenges faced in peripheral or rural laboratories with variable staffing and infrastructure. Certain factors, such as sample transportation delays, environmental conditions, and staff training, impact both methods' effectiveness.[9] Therefore, integrating automated counts with manual verification is often recommended to ensure diagnostic accuracy. To enhance neonatal care in rural tertiary centres, adopting a hybrid approach that leverages automated counting for routine screening, complemented by manual confirmation for complex cases, can optimise resource utilisation and diagnostic precision.[10]Further research focusing on local population haematological parameters, instrument calibration specific to neonatal samples, and cost-benefit analyses can support evidence-based implementation of these methods.

Conclusion

We conclude that both automated and manual techniques of nRBC counting yielded comparable results. Automated analysers, such as the Sysmex XN-1000, can be reliably used as a substitute for manual methods due to their accuracy and speed, helping to reduce the workload in haematological laboratories and improve reliability by reducing human mistakes and prejudice. However, manual microscopy remains the reference method, particularly in cases with extreme nRBC values, abnormal differentials, or analyser flags. A combined approach, integrating automated screening with targeted manual review, appears to be the most efficient strategy for rural tertiary care settings.

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Competing Interests: None

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