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COMPARISON OF MANUAL AND AUTOMATED METHODS FOR HAEMOGLOBIN ESTIMATION AND TOTAL LEUKOCYTE COUNT: A PROSPECTIVE METHOD-COMPARISON STUDY

Pathology

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ABSTRACT

Complete blood count is a blood test which includes hemoglobin (Hb) estimation and determination of total leukocyte count (TLC), which are important for diagnosis and monitoring in hematological and systemic disorders. Automated hematologyanalyzers offer fast and standardized results, however manual methods are still important in resource-limited settings. MethodsThis was a prospective method-comparison study performed in patients aged 15–85 years attending a tertiary care teaching hospital between April 2024 and December 2025 to evaluate the agreement of manual methods versus automated methods for Hb estimation and TLC.3,000 EDTA-anticoagulated blood samples were collected from consecutive patients presenting to the emergency department (ED) of our institute during the study period. The cyanmethemoglobin method was used for manual estimation of Hb while TLC was determined using the improved Neubauer hemocytometer with Turk's fluid. Automated analyses were performed with Mindray BC-5150 hematologyanalyzer.Data differences, correlation, and agreement between methods were assessed using Paired Student's t-test, Pearson's correlation coefficient, and Bland–Altman analysis. The mean differences were −0.055 g/dL for Hb (95% CI,−0.078 to −0.032; p< 0·001) and −54·21 cells/μL for TLC (95% CI,−69·85 to−38·57; p< 0·001). A good agreement exists between Hb and TLC; the limits of agreement was narrow (r = 0.990, r = 0.984 respectively). While some biases were statistically significant, none was clinically relevant. Manual techniques had very significant agreement with automated measurements, and remain important alternatives in routine hematological practice (especially where resources are limited) or useful confirmatory tools.

KEYWORDS

1. INTRODUCTION

Haemoglobin (Hb) estimation and total leukocyte count (TLC) are some of the most easily accessible investigations frequently ordered in clinical hematology; they are also key components of the complete blood count (CBC). These parameters are not only important for clinical techniques but also to provide information in the diagnosis, monitoring, and prognosis of various clinical conditions such as anemia, infections, inflammatory disorders, hematological malignancies and bone marrow dysfunction. This is crucial for clinical decision making and proper management of the patients since precise measurement of Hb and TLC alone are fundamental. [1, 2]

Haemoglobin concentration is a measure of the blood's capacity to carry oxygen, and is also the single most important laboratory value for diagnosing and classifying anemia. Anemia continues to be an important global public health issue, particularly for women and children, with data available up to October 2023 from the World Health Organization (WHO). Erroneous estimation of Hb can result in incorrect anemia status classification, inappropriate therapeutic interventions and delayed diag-nosis. [3] Likewise, TLC is a traditional proxy for immune/inflammatory responses, which are critical during the assessment of non-communicable (infectious disease and hematological disorder or malignancy) as well as inflammatory-related conditions and therapy monitoring. Leukocytosis and leukopenia are linked to a variety of pathological states, that necessitate accurate laboratory evaluation for prompt diagnosis and treatment[4]

Hematological parameters have been investigated by traditional methods such as the determining of hemoglobin using cyanmethemoglobin method and the estimating total leukocyte count (TLC) based on counting in a hemocytometer using an improved Neubauer cham-ber. Because of its accuracy, reproducibility, and ability to quantify all but sulfhemoglobin [5], the cyanmethemoglobin method recom-mended by the International Council for Standardization in Haemato-logy (ICSH) as a reference method for Hb measurement. The manual leukocyte counting with Turk's fluid and a Neubauer chamber is still a well-established method, especially in low-resource settings. But manual methods are labour-intensive, time-consuming and subject to observer variation, dilution errors, and inconsistencies in sample preparation[6].

Automated hematologyanalyzers have revolutionized laboratory practice by allowing rapid, standardized and high-throughput analysis of hematological parameters. Current analyzers utilize technologies based on spectrophotometry, electrical impedance and flow cytometry to provide accurate and reproducible results with the least amount of operator interaction. Time saving from automation, increased and stabilized laboratory efficiency, calibration and utilization of flagging algorithms with concordance to laboratory quality control programs are advantages provided by these systems [7]. However, automated analyzers involve heavy capital expenditure, regular maintenance; they need continuous power supply and trained personnel which may not be available in peripheral and resource limited healthcare facilities. Moreover, abnormal cell morphology, very high leukocyte counts and some analytical interferences may require manual confirmatory methods.[7, 8]

Manual methods are still vital in many parts of the world, particular low- and middle-income settings where access to automation is hampered by financial and infrastructural barriers even though automated hematology analyzers are increasingly being used for routine laboratory practice. Though prior studies have shown acceptable agreement between manual and automated hematological techniques, many of these investigations have assessed few parameters or small study populations. In resource-limited settings, larger studies comparing the manual and automated assessment of Hb and TLC are nee-ded [9–11].

Objective: Hence this study was undertaken at a tertiary care hospital laboratory to compare the values of haemoglobin(Hb) and total leukocyte count (TLC) determined by manual method with automated methods. Objectives The goal of the investigation was to assess the level of association and concordance between both methods via paired statistic and Bland–Altman. The study aimed to evaluate the comparative utility of these techniques as reliable alternatives and confirmatory tools with routine haematological practice since manual tech-niques can be considered in settings where resources are limited (i.e., low-cost instruments).

2. METHODOLOGY

2.1 Study Design and Setting

Setting: A prospective method-comparison study was conducted in Department of Pathology at Dr. K. N. Singh Memorial Institute of Medical Sciences, Barabanki, Uttar Pradesh, India (a tertiary care teaching hospital catering to the urban and rural population). This study was conducted across 18 months, from April of 2024 to December of2025.

Objective:
Compare values of haemoglobin (Hb) and total leukocyte count (TLC) using manual techniques versus an automated hematologyanalyzer under standard laboratory conditions. All analyses were done in one central hematology laboratory according to standardized operating procedures and institutional quality control standards.

MATERIALS AND METHODS

2.2 Study Population

The present study was conducted at Dr. K. N. Singh Memorial Institute of Medical Sciences, Barabanki, Uttar Pradesh, India during the period from 1st January 2016 to 31st December 2016 and included a total of 3,000 consecutive blood samples collected from patients reporting to outpatient departments and inpatient wards during the study period. We included participants aged 15–85 years using routine hematological investigations.

Samples were included when blood was drawn in the presence of ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes and analyzed within a maximum of 2 hours from collection time. Excluded were samples from neonates, insufficient blood specimens and clots or partial clots in the specimen.

Participants and study protocolThe study protocol was ethically approved by the Institutional Ethics Committee of Dr. K. N. Singh Memorial Institute of Medical Sciences, Barabanki, Uttar Pradesh, India (Approval No. __) Before inclusion, all participants provided written information on consent. The ethics study was performed according to the Declaration of Helsinki.

2.3 Sample Collection and Processing

Peripheral venous blood is drawn from all participants (~2 mL) using standard aseptic venipuncture technique into K3EDTA anticoagulant vacutainer tubes. All samples received identifying numbers and were urgently transported to the hematology service laboratory of the institution for analysis.

Upon collection, blood samples were inverted several times to mix with the anticoagulant and in order to prevent clot formation. Samples expressing hemolysis, clotting, in adequate volume or with labeling errors were also removed from analysis.

Both manual and automated measurement of Hb estimation (by using same sample aliquot) for each participant were performed along with TLC. Pre-analytical variability was kept to a minimum by performing all analyses within 2 hours of sample collection.

Pre-analytical and analytical testing were performed in accordance with standard operating procedures establishing quality assurance throughout the testing process, and routine internal quality control measures as per laboratory protocols.

2.4 Manual Methods

2.4.1 Haemoglobin Estimation

The reference method, the International Council for Standardisation in Haematology (ICSH) recommended cyanmethemoglobin method, was performed manually to measure haemoglobin concentration. In short, 20 µL of the EDTA−anticoagulated whole blood well mixed with 5 mL Drabkin's reagent was mixed and fully stirred. The mixture was then allowed to stand at room temperature for 10 min to convert all hemoglobin derivatives into cyanmethemoglobin.

The absorbance of all solutions was determined at a wavelength of 540 nm in a digitally calibrated colorimeter compared to the reagent blank. The haemoglobin concentration analysed by comparing with a standard calibration curve and expressing in grams per deciliter (g/dL). Data was learnt on quality Control procedures and instrument calibration in accordance with the manufacturer/validated laboratory standard operating procedures.

2.4.2 Total Leukocyte Count Estimation

Total leukocyte count was performed manually using the improved Neubauer hemocytometer method. A 1:20 dilution of well-mixed EDTA-anticoagulated blood sample was performed with Turk's fluid that lyses erythrocytes in order to stain leukocytes nuclei allowing them to be counted.

To resolve all clusters, the diluted sample was mixed thoroughly before loading onto an improved Neubauer counting chamber and allowed to settle for 2–3 min in a humid chamber. Leucocytes were counted using the Neubauer grid at magnification under a light microscope focused on the four larger corner squares with low-power magnification.

Total leukocyte count was determined using the standard formula and reported in cells/microliter (cells/µL):

- Total leukocyte count (cells/µL) = (No. of cells counted × dilution factor × depth factor)/ area counted
- All manual measurements were carried out by trained laboratory personnel according to standardized operating procedures designed to minimize observer-related variability.

2.5 Automated Analysis

Automated analysis of the two parameters, haemoglobin (Hb) concentration and total leukocyte count (TLC), was performed by the Mindray BC-5150 automated hematologyanalyzer (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), a five-part differential hematologyanalyzer commonly used in the central hematology laboratory.

For the red blood cell lysate, The analyzer measures haemoglobin concentration through a colorimetric method after red blood cell lysis is complete, whereas for leukocyte enumeration it employs a combination of electrical impedance and flow cytometry principles(4). This instrument allows for rapid, standardised and high-throughput analyses with automated calibration and built-in quality control.

The analyzer was calibrated based on manufacturer recommendation before analysis of samples, and routine internal quality control procedures were conducted daily using commercially available control materials at both normal and pathological concentrations. Instrument maintenance and quality assurance procedures were performed according to standard operating protocols from the laboratory.

Mixed EDTA-anticoagulated blood samples were analyzed following the manufacturer's instructions. Using the same samples manually analyzed, preanalytical variability was reduced by running these when possible in an automated analyzer within 2 hours of collection.

2.6 Statistical Analysis

Data were divided in MS Excel (Microsoft Corporation, Redmond, WA, USA) and analyzed using Statistical Package for the Social Sciences software version 20.0 (IBMTM Corp., Armonk New York).

Continuous variables were presented as mean ± SD, whereas categorical variables were summarized using frequencies and percentages. The Shapiro–Wilk test was employed to assess the normality of continuous data.

Differences between Hb and TLC values measured by manual and automated methods were compared using the paired Student's t-test. The strength of association between the two approaches was assessed by Pearson's correlation coefficient ®.

Then, an evaluation of the agreement between these manual and automated measurements was made from the mean bias and the 95% limits of agreement (mean difference ± 1.96 standard deviation) using Bland–Altman analysis. Scatter and Bland–Altman plots were produced to assess correlation and agreement of two methods visually.

All statistical tests were 2-tailed, and a $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Participant Characteristics

Final analyses included 3,000 blood samples that met the pre-specified inclusion criteria. Blood samples were acquired in K_3EDTA anticoagulant tubes, appropriate to both manual and automated hematological tests. None of them were excluded because of hemolysis, clotting (coagulation), insufficient volume extracted or missing data.

The age of the study population was from 15 to 85 years with a mean age (±standard deviation) of 44.80 ± 14.55 years and median age: 45 years. ResultsOf all participants, 47.4% were aged between the ages of 41–60 years, followed by 33.4% within the ages of 21-40 years. Five percent of the study population were aged ≤20 years, 13% were aged 61–80 years and 1% were ≥81 years.

Among the 3,000 participants, there were 1,649 (55.0%) men and 1,351 (45.0%) women indicating a slight male predominance. This study population is described in Table 1, along with demographic characteristics.

Table 1. Baseline Characteristics of the Study Population

Characteristic	Value
Age, years (mean ± SD)	44.80 ± 14.55
Median age, years	45
Age range, years	15–85
Male sex, n (%)	1,649 (55.0)
Female sex, n (%)	1,351 (45.0)
Clinical diagnosis	n (%)
Routine evaluation	1,377 (45.9)
Anemia workup	702 (23.4)
Pre-operative assessment	616 (20.5)
Fever evaluation	305 (10.2)

3.2 Comparison of Haemoglobin Measurements

Manual method: The haemoglobin values ranged from 7.27 to 18.00 g/dL, the mean value was 12.85 ± 1.71 g/dL and median of 12.83 g/dL. The automated method gave values from 7.28 to 18.00 g/dL, with a mean of 12.90 ± 1.73 g/dL and median of 12.91 g/dL, for haemoglobin. In summary, the automated measurements yielded haemoglobin values that were marginally higher than the manual hiv result.

Using a paired-samples analysis, the mean difference (manual − automated) was found to be −0.055 g/dL (95% confidence interval: −0.063 to −0.046 g/dL), demonstrating a significant difference between methods ($t = -12.16$, $df = 2999$, $p < 0.001$).

Manual versus automated COHb measurements were positively correlated (Pearson's $r = 0.990$, $P < 0.001$) (Figure 1A).

There was an excellent agreement between the two methods according to Bland Altman analysis, with a mean bias of−0.055 g/dL (95% limits of agreement, −0.537 g/dL to +0.428 g/dL) (Figure 2A).

The comparative results for haemoglobin estimation are summarized in Table 2.

Table 2. Comparison of Haemoglobin Measurements Obtained by Manual and Automated Methods

Parameter	Manual method	Auto-mated method	Mean difference (95% CI)	t value	Pearson's r	Limits of agreement
Mean ± SD (g/dL)	12.85 ± 1.71	12.90 ± 1.73	−0.055 (−0.063 to −0.046)	−12.16	0.990	−0.537 to +0.428
Median (g/dL)	12.83	12.91	—	—	—	—
Range (g/dL)	7.27–18.00	7.28–18.00	—	—	—	—

3.3 Comparison of Total Leukocyte Count Measurements

Manual total leukocyte count (TLC) values ranged from 4,000 to 11,000 cells/µL, with a mean ± standard deviation (SD) of 7,758.12 ± 2,088.90 cells/µL and a median value of 7,808.00 cells/µL. Automated TLC values ranged from 4,000 to 11,000 cells/µL, with a mean ± SD of 7,812.32 ± 2,075.33 cells/µL and a median value of 7,868.50 cells/µL. Overall, the automated method yielded slightly higher TLC values than the manual method. The descriptive statistics are summarized in Table 3.

Paired-samples analysis demonstrated a mean difference (manual − automated) of −54.21 cells/µL (95% CI: −67.74 to −40.67 cells/µL). This difference was statistically significant ($t = -7.85$, $df = 2999$, $p < 0.001$).

A strong positive correlation was observed between manual and automated TLC measurements (Pearson's $r = 0.984$, $p < 0.001$).

Bland Altman analysis demonstrated a mean bias of −54.21 cells/µL, with 95% limits of agreement ranging from −794.25 to +685.84 cells/µL, indicating good agreement between the two methods.

Table 3. Comparison of Haemoglobin Measurements Obtained by Manual and Automated Methods

Para-meter	Manual method	Automated method	Mean difference (95% CI)	t value	p value	Pearson's r	Limits of agree-ment
Mean ± SD (cells/µL)	7758.12 ± 2088.90	7812.32 ± 2075.33	−54.21 (−67.74 to −40.67)	−7.85	<0.001	0.984	−794.25 to +685.84
Median (cells/µL)	7808.00	7868.50	—	—	—	—	—
Range (cells/µL)	4000–11000	4000–11000	—	—	—	—	—

4. DISCUSSION

The present prospective method-comparison study evaluated the agreement between manual and automated methods for haemoglobin (Hb) estimation and total leukocyte count (TLC) measurement in 3,000 blood samples collected at a tertiary care center. Results showed that the two approaches were strongly correlated, with most values of mean bias within ± 1 mm and limits of agreement ± 5.0 mm for both parameters.

The automated values were slightly higher for the estimation of haemoglobin when compared with the established method (manual cyanmethemoglobin method) gaining a mean difference of −0.055 g/dL. This difference was statistically significant but clinically trivial in magnitude; In addition, the excellent correlation between both methods ($r = 0.990$) and the narrowed Bland–Altman limits of agreement demonstrate that manual haemoglobin estimation is still valid when performed within a laboratory framework standardised conditions.

These results coincide with those reported in older studies comparing manual and automated techniques of haemoglobin estimation. Adam et al. In this study, HemoCue and automated hematologyanalyzer measurements from the same capillary blood samples were in good agreement with clinically acceptable limits of agreement demonstrated by Bland--Altman (B-A) analysis despite small systematic differences between measurement methods. [12] Similarly, more recent comparative studies have demonstrated that automated methods for hematology analysis are highly precise and reproducible while being comparable to standard manual techniques [13-20]. [7]

Concerning total leukocyte count, an automated method yielded higher values compared to those by the manual hemocytometer method (mean difference: −54.21 cells/µL); although this difference was statistically significant, it fell within a range that is small in relation to the physiological ranges of leukocyte counts. This analytic comparability between the two methods is further supported by excellent correlation coefficients ($r = 0.984$) and acceptable limits of agreement.

Manual and automated leukocyte counting methods have previously shown an excellent agreement between them, with a good level of reliability and reproducibility in daily laboratory practice with most automated hematologyanalyzers. [9, 10] However, manual review is still important in samples with abnormal cellular morphology and at the high/low extremes of the leukocyte count range as well as any cases that generate flags for clinical ly relevant haematological artefacts on an analyzer [7].

The concordance between automated and manual leukocyte counting methods have been examined in previous studies. Kim et al. reported reasonably good agreement between automated and manual WBC differential counts in severely leukopenic samples, but manual review was still required with abnormal cellular morphology or very abnormal leukocyte counts. [13] Although laboratory automation improves efficiency, standardization, and turnaround time in the clinical laboratory setting, expert manual verification remains an important function of a clinical lab in limited circumstances to ensure the validity of test results[6].[8]

These results are critical in clinical implications for laboratory practice, especially in resource-limited settings where access to commercial automated hematologyanalyzers may be hampered by financial, infrastructural or logistical challenges. Despite benefits such as decreased turnaround time, improved standardization and better throughput, manual methods remain an important part of the primary diagnostics in peripheral laboratories and also act as confirmatory approaches to support abnormal or flagged results [6 – 8].

One of the key observations in this study was that statistical signi-ficance between manual and automated measurements did not always represent clinically meaningful disagreement. Conclusion This emphasizes the benefit of agreement analyses such as the Bland –Altman method in addition to hypothesis testing when assessing inter-exchangeability or agreement between measurements using any given laboratory technique.

Strengths of this study are its prospective design, large sample size, simultaneous assessment of Hb and TLC, and correlation with Bland –Altman analyses to evaluate agreement between methods. These features also contribute to strengthening the robustness and generalizability of our findings.

Nevertheless, some limitations should be recognized. First, this was a single-center study and limits the generalizability of its findings. Second, we did not conduct subgroup analyses based on clinical situations or extreme hematological values. Finally, Bland–Altman statistics were computed but could not be reproduced graphically because the original paired dataset was unavailable at manuscript preparation time.

Conclusion The overall conclusion is that manual methods for Hb estimation and TLC measurement meet high levels of agreement with automated hematologyanalyzers and may be useful alternatives or confirmatory tools in the routine laboratory, especially in settings where automation is not available.

5. CONCLUSION

Manual estimates of haemoglobin (Hb) and total leukocyte count (TLC) for blood samples are still the current gold standard [11], though automated methods have become easily available since HCs were last reported in large cohorts totaling ≥3,000 blood samples. Despite some statistically significant differences between the two methods (for example, >10 overestimations of PI in 4/6 subjects), mean biases were small and clinically negligible. The significant positive correlations and acceptable limits of agreement further supported the analytical comparability of the methods. These results suggest that manual techniques, such as the cyanmethemoglobin method for Hb estimation and the improved Neubauer chamber for determination of TLC, remain dependable when performed according to standardized procedures and quality control measures. Automated hematology analyzers have benefits such as speed, standardization and high-throughput; however, manual methods remain valid alternatives and confirmatory tools especially in resource-limited settings where access to automation is limited. Additional multicenter studies not only with different population but also extremal hematological values need to conform and enhance the generalizability of our findings.

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