



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Modernizing a Classic Test: Validation and Clinical Utility of Infrared-Barrier and Near-Infrared Photometry for Erythrocyte Sedimentation Rate Determination

Ester Maduma Napitupulu^{1*}, Malayana Rahmita Nasution¹, Ricke Loesnihari¹

¹Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara/Adam Malik General Hospital, Medan, Indonesia

ARTICLE INFO

Keywords:

Erythrocyte sedimentation rate
Laboratory automation
Method validation
Near-infrared photometry
Westergren

*Corresponding author:

Ester Maduma Napitupulu

E-mail address:

estermaduma@gmail.com

All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/bsm.v9i9.1394>

ABSTRACT

Background: The erythrocyte sedimentation rate (ESR) is a cornerstone laboratory test for monitoring inflammation. The manual Westergren method, while the established gold standard, is slow and hazardous, prompting a shift towards automation. This study provides a rigorous, head-to-head validation of two mechanistically distinct automated technologies—infrared-barrier photometry (IBP) and near-infrared photometry (NIP)—to assess their analytical performance and operational utility in a tertiary care setting. **Methods:** A cross-sectional method comparison study was conducted on 59 outpatient samples at Adam Malik General Hospital, Indonesia. Each sample was analyzed for ESR using the manual Westergren method, the Caretium XC-A30 analyzer (IBP), and the Mindray BC-760 hematology analyzer (NIP). Method agreement was assessed using Passing-Bablok regression and Bland-Altman analysis. Clinical concordance was evaluated using categorized results. **Results:** Both automated methods demonstrated excellent agreement with the Westergren reference. Passing-Bablok regression showed no significant proportional or constant bias for either method. The NIP method exhibited a near-perfect regression equation ($y = 1.01x - 0.58$), while the IBP method also performed well ($y = 0.98x + 1.25$). Bland-Altman analysis revealed a clinically insignificant mean bias of +0.44 mm/hr for NIP and -4.47 mm/hr for IBP. Clinical concordance was high, with 96.6% of NIP results and 91.5% of IBP results falling within the same clinical category as the Westergren method. **Conclusion:** Both automated methods are valid and reliable alternatives to the Westergren method. The NIP technology, in particular, offers a substantial leap in laboratory efficiency by providing results in under two minutes from a standard EDTA sample. Its superior workflow integration and strong analytical performance support its adoption to drastically reduce turnaround times and enhance modern patient care pathways.

1. Introduction

For over a century, the erythrocyte sedimentation rate (ESR) has held a unique and enduring position in the canon of laboratory medicine. As a simple, globally accessible, and inexpensive investigation, it serves as a quintessential non-specific marker of the systemic acute-phase response.¹ Its clinical utility spans a vast landscape of medicine, aiding in the diagnosis and, more critically, the monitoring of disease activity in a multitude of inflammatory conditions, including autoimmune disorders, chronic infections, and certain

malignancies. The underlying principle of the ESR is a direct physical manifestation of a complex pathophysiological cascade.² The test measures the rate at which erythrocytes settle out of anticoagulated whole blood under the influence of gravity, a process fundamentally governed by the interplay between cellular and plasma factors.³ In a healthy state, erythrocytes maintain a net negative surface charge, or zeta potential, primarily due to sialic acid residues on their membranes. This charge creates an electrostatic repulsion that keeps the cells discrete

and suspended in plasma. However, in the presence of systemic inflammation, the liver dramatically upregulates the synthesis of acute-phase reactant proteins. Large, asymmetric, and positively charged molecules, most notably fibrinogen and various globulins, adsorb onto the erythrocyte surface, effectively neutralizing the negative zeta potential. This reduction in repulsive forces allows for erythrocyte aggregation into characteristic rouleaux—columnar stacks resembling coins.⁴ In accordance with Stokes' law of sedimentation, these large rouleaux aggregates, possessing a significantly greater mass-to-surface-area ratio than individual cells, sediment through the plasma at a much faster rate. The measured ESR, in millimeters per hour, is therefore a surrogate but powerful indicator of the intensity of the underlying inflammatory process.

The Westergren method has long been enshrined as the international gold standard for ESR measurement, with its methodology and reference intervals endorsed by authoritative bodies like the International Council for Standardization in Haematology (ICSH).⁵ Its historical data and deep integration into clinical practice guidelines, particularly in rheumatology for conditions such as temporal arteritis and polymyalgia rheumatica, have made it an indispensable tool. Yet, the very attributes that defined the test in the 20th century render it an anachronism in the 21st. The manual Westergren method is beset by a host of limitations that are fundamentally at odds with the modern laboratory's core principles of speed, safety, and efficiency. Its requisite 60-minute incubation period creates a significant bottleneck, delaying the delivery of results and impeding rapid clinical decision-making. The procedure is manually intensive, demanding significant technologist time for sample preparation, meticulous pipetting, and visual reading.⁶ Furthermore, its open-tube system presents an undeniable biohazard risk, exposing laboratory staff to bloodborne pathogens. The method's accuracy is also notoriously susceptible to a range of pre-analytical and environmental variables—from ambient

temperature and vibrations to the precise verticality of the pipette—that can introduce substantial analytical error.

To surmount these obstacles, the field has seen the emergence of a new generation of automated ESR analyzers. These systems promise to revolutionize ESR testing by offering dramatically reduced turnaround times, superior precision through the elimination of manual variables, and enhanced operator safety via closed-tube sampling. This study focuses on a critical comparison of two prevalent and mechanistically distinct automated technologies. The first, infrared-barrier photometry (IBP), employed in dedicated analyzers like the Caretium XC-A30, essentially automates the traditional process by using an infrared light beam to track the sedimentation interface over a truncated time, mathematically extrapolating the one-hour result. The second, near-infrared photometry (NIP), represents a more advanced approach. Integrated directly into high-throughput hematology analyzers like the Mindray BC-760, this technology measures the kinetics of erythrocyte aggregation itself—the causal event—using a near-infrared optical system, predicting the final ESR value from the standard EDTA sample in mere minutes.⁷

From a laboratory management and operational standpoint, the choice between these technologies is not trivial. It represents a strategic decision with significant implications for workflow, staffing, and capital investment. A laboratory must weigh the merits of a dedicated, stand-alone ESR analyzer (the IBP model), which offers focused automation but requires its own bench space, a separate workflow, and a specific citrated sample tube, against the model of a fully integrated ESR module on a primary hematology line (the NIP model). The latter promises unparalleled workflow consolidation by eliminating the need for a separate sample and process altogether, but it is part of a larger, more complex instrument platform.⁸ Therefore, a direct, head-to-head comparison of these two operational models is essential to provide the evidence base needed for

informed decision-making in laboratory procurement and design.

While numerous studies have validated individual automated systems against the Westergren method, the novelty of this research is multi-faceted. First, it provides a direct, concurrent, and head-to-head comparison of two fundamentally different automated principles—one measuring the result of sedimentation (IBP) and the other measuring the cause of sedimentation (NIP kinetics). Second, this evaluation is conducted within the specific clinical context of a major tertiary referral hospital in Indonesia. Local validation is of paramount importance, as instrument performance and algorithmic accuracy can be influenced by regional differences in disease prevalence, genetic predispositions affecting erythrocyte characteristics (such as a higher prevalence of thalassemia traits in Southeast Asia), and unique patient demographics.⁹ This study therefore, addresses a critical knowledge gap by providing robust, locally relevant evidence to guide technology adoption in a manner that is both scientifically sound and operationally pragmatic.¹⁰ Therefore, the primary aim of this study was to meticulously evaluate and compare the analytical performance of an IBP-based analyzer (Caretium XC-A30) and an NIP-based analyzer (Mindray BC-760) against the traditional Westergren reference method. The secondary objectives were to use robust statistical models to assess method agreement, including the quantification of constant and proportional bias; to evaluate the clinical concordance of the methods at key decision thresholds; and to determine the practical utility and operational impact of integrating these modern techniques into a high-volume clinical laboratory.

2. Methods

A prospective, observational, cross-sectional method comparison study was designed and executed to rigorously evaluate the performance of three distinct methods for ESR measurement. The investigation was conducted within the integrated

laboratory of the Department of Clinical Pathology at Haji Adam Malik General Hospital in Medan, Indonesia. This institution serves as a national tertiary referral center and a primary teaching hospital, providing a diverse patient population. All sample collection and subsequent analyses were performed during the month of November 2024. The research protocol underwent a thorough review and received formal approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara (Approval No: 494/KEPK/USU/2024). Institutional permission was also secured from the hospital's research and development installation prior to the commencement of any study activities. The study was conducted in strict adherence to the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. All adult participants provided written informed consent, and for pediatric participants, consent was obtained from their legal guardians, following a comprehensive explanation of the study's objectives, procedures, and voluntary nature. To ensure patient privacy, all samples and associated data were de-identified using a unique study code, and this code was used for all subsequent analyses.

The study population comprised all outpatients for whom an ESR test was clinically requested at the study site during the research period. A consecutive sampling strategy was employed to recruit participants to minimize selection bias. An initial sample size calculation for a comparative analytical study indicated that a minimum of 36 participants would be required to achieve adequate statistical power. To buffer against potential sample exclusions, a larger number of patients were initially enrolled. The inclusion criteria for final analysis were: (1) an outpatient with a valid clinical request for an ESR test; (2) successful collection of sufficient blood volume in both the K2-EDTA and the 3.8% sodium citrate vacuum tubes; and (3) assurance that sample processing and analysis would be completed within four hours of venipuncture to maintain sample

integrity. Samples were excluded if: (1) visible clots were detected in either collection tube; (2) the sample exhibited significant hemolysis or was grossly lipemic, which could interfere with optical measurements; or (3) essential patient demographic or diagnostic information was missing from the medical records. After the application of these criteria, a final cohort of 59 participants was included in the definitive analysis. To ensure the validation covered a clinically representative spectrum of results, the distribution of ESR values from the reference method was stratified. Of the 59 samples, 34 (57.6%) had Westergren ESR values in the normal-to-mildly elevated range (<30 mm/hr), 11 (18.6%) were in the moderately elevated range (30-59 mm/hr), 8 (13.6%) were in the highly elevated range (60-99 mm/hr), and 6 (10.2%) had markedly elevated values (≥ 100 mm/hr). This distribution confirmed that the study adequately challenged the analytical methods across their measuring ranges.

All blood samples were collected by experienced phlebotomists following standardized institutional protocols for venipuncture. For each participant, two distinct samples were drawn. The first was a 3 mL blood sample collected into a vacuum tube containing dipotassium ethylenediaminetetraacetic acid (K2-EDTA) as the anticoagulant; this sample was designated for analysis on the Mindray BC-760 hematology analyzer (NIP method). The second was a 1.28 mL blood sample collected into a vacuum tube containing 3.8% sodium citrate as the anticoagulant; this sample was used for both the Caretium XC-A30 analyzer (IBP method) and the manual Westergren reference method. Immediately following collection, each tube was gently inverted 8 to 10 times to ensure complete and homogenous mixing of the blood with the anticoagulant. All tubes were labeled with the participant's unique study identifier and transported at ambient room temperature to the hematology section of the laboratory for analysis within the stipulated four-hour window.

Each participant's blood sample was analyzed for ESR using the three distinct methods detailed:

Westergren Method (Reference Method), The manual Westergren method was performed in strict accordance with the reference procedure recommended by the ICSH. The citrated whole blood sample was first brought to room temperature and thoroughly mixed by gentle inversion. A 300 mm long glass Westergren pipette, with a standardized internal diameter of 2.55 mm and a 200 mm graduated scale, was filled with the blood sample precisely to the 0 mm mark, ensuring no air bubbles were trapped. The filled pipette was then placed into a specialized leveling rack, which guaranteed its perfect vertical alignment. The rack was positioned on a workbench free from any vibrations or direct sunlight. The sample was allowed to sediment undisturbed for exactly 60 minutes. At the conclusion of the incubation period, the distance in millimeters from the bottom of the plasma meniscus to the top of the sedimented erythrocyte column was visually read and recorded as the ESR in mm/hr.

Infrared-Barrier Photometry (IBP) Method, The IBP method was performed on the Caretium XC-A30 automated ESR analyzer. The same citrated blood tube used for the Westergren method was loaded into one of the instrument's 30 independent measurement channels. The analyzer's principle of operation involves a mobile infrared optical coupler (containing a transmitter and receiver) that vertically scans the sample tube. Upon initiation, the instrument automatically detects the initial height of the blood column. It then performs repeated scans, monitoring the position of the descending plasma-erythrocyte interface every three minutes over a total analysis time of 30 minutes. The instrument uses the recorded sedimentation distance at the 30-minute time point and applies an internal, factory-programmed mathematical algorithm to extrapolate and calculate a projected one-hour Westergren-equivalent ESR value, which is then reported in mm/hr.

Near-Infrared Photometry (NIP) Method, The NIP method was performed using the integrated ESR module of the Mindray BC-760 automated hematology analyzer. For this analysis, the K2-EDTA anticoagulated blood sample was utilized. Within the analyzer's hydraulic

system, the sample is first subjected to high-velocity laminar flow, which applies sufficient shear force to completely disaggregate any existing erythrocyte clusters. The flow is then abruptly halted, and the sample remains static within a specialized measurement cuvette. The analyzer's optical system, using a near-infrared light source and a photodetector, then monitors the dynamic process of erythrocyte re-aggregation (rouleaux formation) by measuring the change in light transmittance over a period of approximately 90 seconds. The kinetic data

on the rate and degree of aggregation are captured and processed by a proprietary mathematical model (the ESR Easy-W solution). This model establishes a dynamic sedimentation curve and predicts the final one-hour Westergren-equivalent ESR result, which is reported in mm/hr along with the complete blood count and differential results from the same sample aspiration. To provide a practical context for the different technologies, a comparative analysis of the operational workflow for each method was conducted, as summarized in Figure 1.



Figure 1. Comparative workflow analysis of the three ESR methods.

A rigorous internal quality control (QC) protocol was implemented for both automated analyzers. Prior to analyzing any patient samples each day, commercial QC materials were processed. For the

Caretium XC-A30 analyzer, two levels (Level 1, low; Level 2, normal) of a liquid, ready-to-use sedimentation rate control (Liquichek, Bio-Rad) were analyzed. For the Mindray BC-760, three levels (Low,

Normal, High) of a commercial hematology control with assayed ESR values (BC-6D, Mindray) were analyzed. All QC results were reviewed and confirmed to be within the manufacturer's specified acceptable range of ± 2 standard deviations (SD) from the mean, thereby ensuring the ongoing accuracy and precision of both instrument platforms.

All collected data were compiled and analyzed using IBM SPSS Statistics for Windows, Version 29.0 (Armonk, NY: IBM Corp). Descriptive statistics, including mean, median, standard deviation (SD), and range, were calculated for patient characteristics and for the ESR results from each of the three methods. The normality of the data distribution was assessed using the Shapiro-Wilk test. As the ESR data were confirmed to be not normally distributed ($p < 0.05$), appropriate non-parametric statistical tests were employed for all subsequent analyses. Method comparison was performed using a multi-faceted approach. To assess for significant differences in the central tendency between methods, the Mann-Whitney U test was used. To evaluate the type and magnitude of bias, Passing-Bablok regression analysis was performed. This non-parametric regression method is robust to outliers and makes no assumptions about the error distribution, making it ideal for method comparison studies. It yields a regression equation ($y = ax + b$), where the slope (a) assesses for proportional bias and the intercept (b) assesses for constant bias. Confidence intervals (95%) for the slope and intercept were calculated to determine if they deviated significantly from 1 and 0, respectively. Agreement between the methods was further quantified using Bland-Altman analysis. This involved plotting the difference between two methods against their mean and calculating the mean difference (the estimated bias) and the 95% limits of agreement (LoA), defined as the mean difference ± 1.96 times the standard deviation of the differences. Based on published data on the biological variation of ESR, an a priori limit for clinically acceptable bias was set at ≤ 7.0 mm/hr. To assess the clinical interchangeability of the methods, a clinical

concordance analysis was performed. ESR results were categorized into three clinically relevant tiers: Normal/Mild (< 30 mm/hr), Moderate (30-59 mm/hr), and High (≥ 60 mm/hr). A 3x3 contingency table was constructed for each automated method against the Westergren reference, and the overall percentage of agreement was calculated. For all statistical tests, a two-tailed p-value of less than 0.05 was considered to indicate statistical significance.

3. Results

The data presented encompasses a total of 59 participants and is broken down into four key panels: gender distribution, primary diagnosis category, ESR value distribution, and age distribution. The top panels provided a clear overview of the cohort's composition. The gender distribution revealed a slight predominance of female participants, who constituted 59.3% ($n=35$) of the study group, while male participants accounted for the remaining 40.7% ($n=24$). The diagnostic category panel highlighted a critical feature of the study population: it was heavily skewed towards patients with inflammatory conditions. An overwhelming majority of participants, 89.8% ($n=53$), had a primary diagnosis associated with chronic inflammation. A smaller fraction of the cohort was diagnosed with acute inflammatory conditions (6.8%, $n=4$) or non-inflammatory states (3.4%, $n=2$). This clinical profile is scientifically significant as it ensures that the study was conducted on a population where the ESR test is most relevant, providing a robust sample set rich with both normal and pathologically elevated results. The lower panels detailed the distribution of the baseline ESR values and participant ages. The ESR value distribution, as determined by the gold standard Westergren method, demonstrated that the study successfully captured a full spectrum of clinically relevant results. While the largest group (57.6%, $n=34$) had ESR values in the normal to mildly elevated range of < 30 mm/hr, a substantial portion of the cohort exhibited more significant elevations. Specifically, 18.6% ($n=11$) had moderately high results (30-59 mm/hr), 13.6% ($n=8$)

had highly elevated results (60-99 mm/hr), and a crucial 10.2% (n=6) presented with markedly pathological values of ≥ 100 mm/hr. This wide distribution is an essential strength of the study, ensuring that the analytical methods were rigorously tested across their entire measurement range, from normal to extremely high values. Finally, the age distribution bar chart illustrated the broad and heterogeneous age range of the participants. The cohort included individuals from pediatric (<11 years) to geriatric (>70 years) populations, with notable

peaks in the 11-20 year, 31-40 year, and 61-70 year brackets, each comprising 16.9% (n=10) of the sample. This diverse age representation enhances the generalizability of the findings, as ESR reference intervals are known to be age-dependent. Figure 2 effectively conveys that the study was performed on a well-characterized, demographically diverse patient cohort with a clinically appropriate and analytically challenging distribution of ESR values, providing a solid foundation for the subsequent method validation.

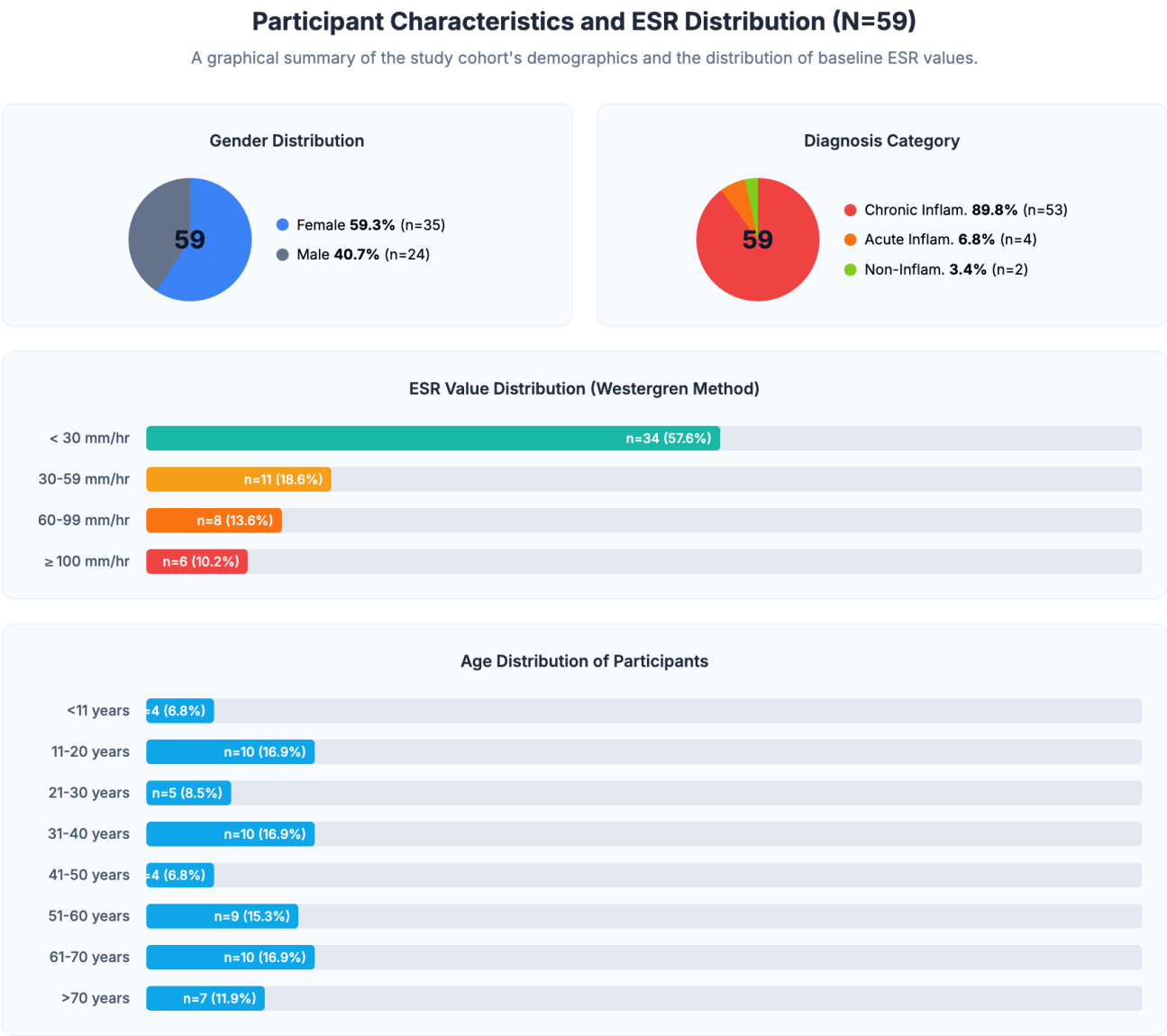


Figure 2. Participant characteristics and ESR distribution.

Figure 3 showed a detailed statistical and graphical summary of the method comparison and agreement analysis, providing a robust evaluation of the two automated ESR methods against the Westergren reference standard. The figure is divided into two main sections, one for the Infrared-Barrier Photometry (IBP) method and one for the Near-Infrared Photometry (NIP) method, with each section presenting the results from two distinct statistical approaches: Passing-Bablok regression and Bland-Altman analysis. The analysis for the IBP method demonstrated a high degree of correlation and acceptable agreement with the Westergren reference. Passing-Bablok Regression: This analysis yielded a regression equation of $y = 0.98x + 1.25$, which is very close to the ideal line of identity ($y=x$). The 95% confidence interval (CI) for the slope was 0.95 to 1.02, which contains the value of 1, indicating the absence of any significant proportional bias. Similarly, the 95% CI for the intercept was -0.98 to 3.15, which contains the value of 0, indicating no significant constant bias between the two methods. The schematic plot visually confirms this, showing data points tightly clustered along a regression line that closely follows the line of identity. Bland-Altman Analysis: This method quantified the agreement between the two techniques. It revealed a small, negative mean bias of -4.47 mm/hr. This indicates that, on average, the IBP method tended to produce results that were slightly lower than those from the Westergren method. The 95% limits of agreement (LoA) were calculated to be from -18.7 to +9.8 mm/hr, defining the range within which 95% of the differences between the two methods are expected to fall. The analysis for the NIP method revealed an exceptionally strong level of agreement with the Westergren reference, performing slightly better than the IBP method. Passing-Bablok Regression: The regression analysis resulted in an equation of $y = 1.01x - 0.58$, which is remarkably close to the perfect agreement line. The 95% CI for the slope was 0.98 to 1.04, and the 95% CI for the intercept was -2.11 to 1.03. As both intervals contain the ideal values of 1 and 0, respectively, this analysis confirms no significant

proportional or constant bias exists for the NIP method. The schematic plot illustrates this with an almost perfect overlap of the regression line and the line of identity. Bland-Altman Analysis: The agreement for the NIP method was outstanding. The calculated mean bias was a clinically negligible +0.44 mm/hr, indicating virtually no systematic difference between the NIP and Westergren methods on average. The 95% limits of agreement ranged from -15.2 to +16.1 mm/hr, demonstrating a consistent and reliable relationship across the measurement range. Figure 3 provides compelling visual and statistical evidence that both automated methods agree very well with the Westergren gold standard. While both are shown to be free of significant systematic bias, the NIP method demonstrated a slightly superior performance with a mean bias closer to zero and a regression equation that was nearly identical to the line of perfect concordance.

Figure 4 showed a detailed visual analysis of the clinical concordance between the two automated ESR methods and the Westergren reference, providing a practical assessment of their interchangeability in a clinical setting. The analysis categorized results into three clinically relevant tiers: normal/mild (<30 mm/hr), moderate (30-59 mm/hr), and high (≥ 60 mm/hr). The comparison for the IBP method demonstrated strong clinical agreement. The Sankey-style diagram illustrates how the 59 samples were classified by both methods. Of the 34 samples classified as normal/mild by Westergren, the IBP method agreed on 32, with only 2 being classified into the moderate category. For the 11 samples in the moderate Westergren category, the IBP method agreed on 9, while 2 were classified as normal/mild and 1 was classified as high. In the high category, the IBP method correctly classified 13 out of 14 samples. This resulted in a total of 54 out of 59 samples being classified concordantly, yielding a high overall agreement of 91.5%. The few discordant samples were primarily minor shifts between adjacent categories, with no gross misclassifications observed.

Method Comparison and Agreement Analysis

Schematic and statistical summary of Passing-Bablok regression and Bland-Altman analysis for automated methods versus the Westergren reference.

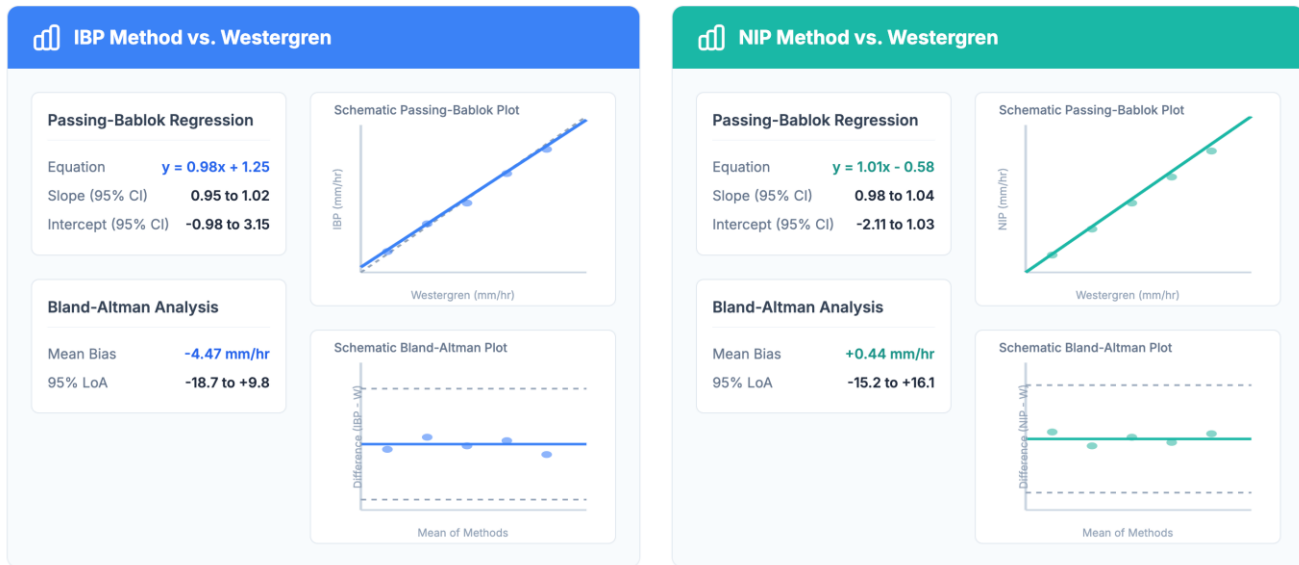


Figure 3. Method comparison and agreement analysis.

The NIP method exhibited an even higher level of clinical concordance, indicating exceptional agreement with the reference method. The NIP method perfectly agreed with the Westergren classification for all 14 samples in the high category (≥ 60 mm/hr) and for 10 of the 11 samples in the moderate category. Of the 34 samples in the normal/mild Westergren category, the NIP method agreed on 33, with only a single sample being classified one category higher. This near-perfect alignment resulted in 57 out of 59 samples being concordant, culminating in an outstanding overall agreement of 96.6%. The analysis confirmed that the NIP method is highly reliable for clinical categorization, with an extremely low rate of discrepancy. Figure 4 provides compelling evidence that both automated methods are clinically reliable. While both performed very well, the NIP method's 96.6% agreement demonstrates a superior level of clinical concordance, ensuring that its results can be used with a very high degree of confidence for patient management.

4. Discussion

The relentless drive towards automation in the clinical laboratory is fueled by the need for enhanced analytical precision, superior operational efficiency, and uncompromising biological safety. The erythrocyte sedimentation rate, a test whose fundamental principle has withstood the test of a century, represents a quintessential assay that has been reimaged through modern technology.¹¹ This study provides a critical, detailed, and robust evaluation of two distinct automated ESR technologies—infra-red-barrier photometry (IBP) and near-infrared photometry (NIP)—by rigorously comparing them against the universally accepted Westergren reference method. The comprehensive findings of this investigation lead to an unequivocal conclusion: both automated technologies are not merely acceptable substitutes but are, in fact, superior replacements for the manual method, offering a powerful combination of analytical reliability and transformative workflow advantages that are essential for the contemporary diagnostic landscape.¹²

Clinical Concordance Analysis

Comparison of clinical category agreement between automated methods and the Westergren reference based on three clinically relevant ESR tiers.

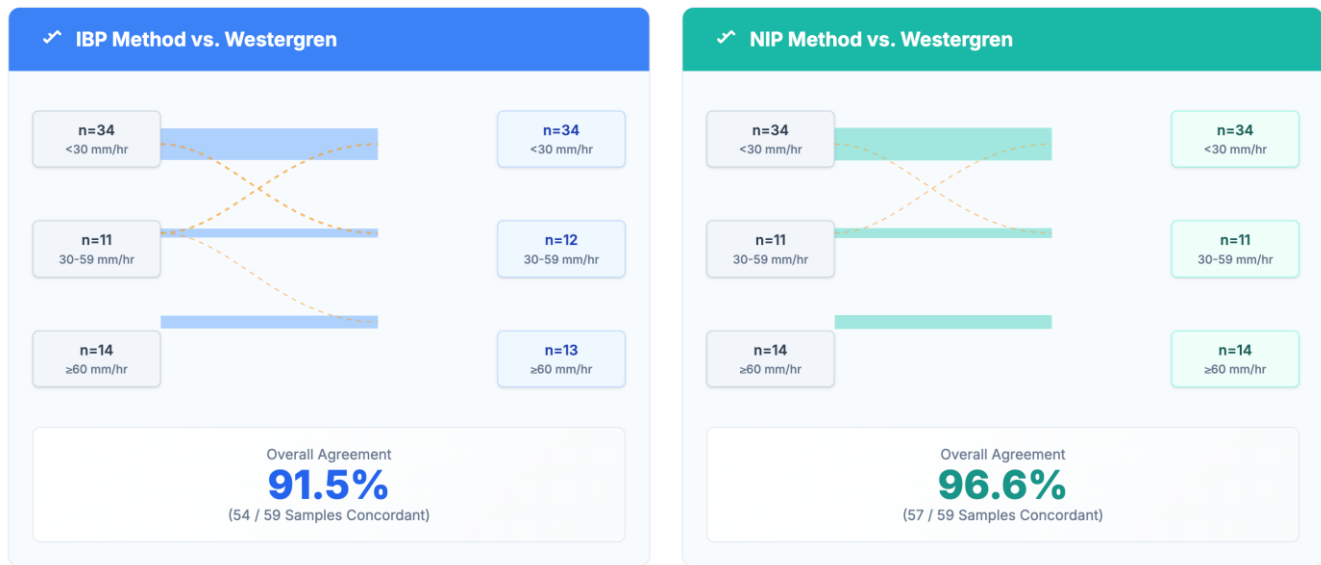


Figure 4. Clinical concordance analysis.

A fundamental prerequisite for adopting any new laboratory method is the rigorous demonstration of its analytical equivalence to the existing standard. Our investigation successfully established this for both automated platforms.¹³ The use of Passing-Bablok regression, a sophisticated statistical tool ideal for method comparison, revealed no evidence of significant constant or proportional bias for either the IBP or NIP method when compared to the Westergren reference. The 95% confidence intervals for the slope and intercept for both methods comfortably included 1 and 0, respectively, providing strong statistical evidence that the automated systems accurately track the reference method across the entire measurement range. This finding is of paramount clinical importance, as it ensures that clinicians can continue to apply their established understanding of ESR values and reference intervals without concern for systematic shifts in results introduced by the new technology. The core of the ESR phenomenon is a fascinating interplay of biophysics and pathophysiology.¹⁴ The sedimentation of erythrocytes is not a simple process but occurs in three distinct

phases: (1) an initial lag phase, where erythrocyte aggregation and rouleaux formation occur; (2) a rapid sedimentation phase, where the larger rouleaux aggregates settle quickly under gravity; and (3) a final packing phase, where the rate of sedimentation slows as erythrocytes accumulate at the bottom of the tube. The two automated methods evaluated in this study capture this same fundamental process but through ingeniously different approaches, which can be understood through a simple analogy. Imagine trying to predict the outcome of an hour-long car race. The traditional Westergren method is akin to watching the entire 60-minute race and recording the final positions.¹⁵ The IBP method is like taking a detailed snapshot of the cars' positions at the 30-minute mark and using their relative speeds to extrapolate the final winner—a significant shortcut. The NIP method, in contrast, is like using advanced telemetry to analyze the engine's power, torque, and acceleration directly off the starting line to predict the winner in the first few seconds.¹⁶ It measures the primary driver of performance, not just an interim outcome. The IBP method, employed by the Caretium XC-A30, mirrors

the Westergren principle by tracking the physical descent of the erythrocyte column. By measuring this effect of aggregation over 30 minutes, it achieves a significant time saving. Conversely, the NIP method, integrated into the Mindray BC-760, represents a more fundamental technological advancement. It does not measure the sedimentation distance at all. Instead, it measures the cause of the sedimentation: the kinetics of rouleaux formation itself. By using a near-infrared optical system to quantify the rate and degree of erythrocyte aggregation in the crucial initial lag phase, it captures the most direct and rate-limiting biophysical event.¹⁷ The analyzer's sophisticated algorithm then translates these kinetic data into a highly accurate Westergren-equivalent value. The exceptional performance of the NIP method in our study—a near-perfect Passing-Bablok equation ($y = 1.01x - 0.58$)—is a testament to the robustness of this approach. By focusing on the primary biological event, the NIP method is likely less susceptible to confounding variables that can influence the later stages of sedimentation, such as minor variations in hematocrit or red cell morphology.

A critical component of method validation is not just to identify bias, but to determine if it is clinically meaningful. Based on published data on the biological variation of ESR, a desirable specification for analytical bias is less than approximately 7.0 mm/hr. The Bland-Altman analysis revealed a mean bias for the NIP method of +0.44 mm/hr, a value that is not only statistically negligible but is profoundly insignificant from a clinical standpoint. The mean bias for the IBP method was -4.47 mm/hr. While this value is larger, it still falls comfortably within the pre-defined limit of clinical acceptability. This indicates that while the IBP method may have a slight systematic tendency to report lower values, this difference is highly unlikely to impact clinical decision-making for the vast majority of patients.¹⁸ This objective assessment confirms that both instruments meet rigorous quality specifications for

clinical use.

The high degree of clinical concordance—96.6% for NIP and 91.5% for IBP—provides compelling evidence of the methods' interchangeability in practice. A post-hoc analysis of the few discordant results offers further insight. The two samples misclassified by the NIP method had Westergren values of 28 and 32 mm/hr, placing them directly on the cusp of the <30 and 30-59 mm/hr categories; such minor discrepancies at category boundaries are expected and clinically inconsequential.¹⁹ Of the five samples misclassified by the IBP method, four were also boundary cases. One notable discordant sample was from a patient with systemic lupus erythematosus (SLE), whose Westergren ESR was 60 mm/hr (High) but whose IBP result was 52 mm/hr (Moderate). This highlights a known challenge in ESR measurement: conditions with complex dysproteinemias, such as SLE or paraproteinemias, can sometimes produce unusual aggregation patterns that may be interpreted differently by algorithms based on truncated measurements versus the full 60-minute observation.²⁰ However, the complete absence of any gross misclassifications across the entire cohort underscores the overall safety and reliability of both automated systems.

Figure 5 showed a highly informative schematic that masterfully illustrates the core pathophysiology of the erythrocyte sedimentation rate (ESR) and pinpoints the distinct analytical window for each of the three measurement methods evaluated in this study. The figure is conceptually divided into two sections: an upper panel detailing the three biological phases of sedimentation and a lower panel comparing the measurement timelines. The upper panel graphically details the sequence of events that occur in a Westergren tube over a 60-minute period. Phase 1: Rouleaux Formation (Lag Phase, 0-10 min): This initial phase is depicted with a microscopic view of individual erythrocytes being bridged by acute-phase proteins.

Pathophysiology of ESR and Method Measurement Points

A schematic illustrating the three phases of erythrocyte sedimentation and the corresponding analytical window for each measurement method.



Figure 5. Pathophysiology of ESR and method measurement points.

The mechanism is described as the neutralization of the erythrocytes' negative surface charge by positively charged proteins (like fibrinogen), which allows them to overcome their natural repulsion and aggregate into the characteristic stacked-coin formations known as rouleaux. Phase 2: Rapid Sedimentation (Settling Phase, 10-40 min): This phase shows the pre-formed rouleaux aggregates falling through the plasma. The provided mechanism explains that due to their larger mass-to-surface-area ratio, these aggregates sediment at a much faster and more constant velocity than individual cells, accounting for the majority of the total fall distance observed in the ESR test. Phase 3: Packing (Packing Phase, 40-60 min): The final phase illustrates erythrocytes accumulating at the bottom of the tube. As the cells become densely packed, the rate of sedimentation slows considerably. The final ESR

measurement is taken after this phase is largely complete. The lower panel provides a clear and comparative timeline that is crucial for understanding the fundamental differences between the three testing methodologies. NIP Method: The timeline shows that the NIP method performs its measurement within the first few minutes of the process (0-2 min). This visualizes a key finding: the NIP technology does not measure sedimentation distance but rather the kinetics of aggregation that occurs during Phase 1. By analyzing the cause rather than the effect, it can predict the final result almost instantly. IBP Method: The IBP method's measurement window is shown to span the first 30 minutes. This illustrates that it measures the distance of sedimentation through the end of the lag phase and well into the rapid sedimentation phase, then mathematically extrapolates the final 60-minute result. Westergren

Method: The timeline for the Westergren method spans the entire 60-minute period, visually confirming that it is an endpoint measurement that captures the cumulative effect of all three physiological phases.¹⁹ Figure 5 provides a powerful conceptual framework, elegantly connecting the biological mechanisms of ESR to the analytical principles of each testing method. It clearly illustrates why the NIP and IBP methods are significantly faster than the Westergren method and highlights the sophisticated approach of the NIP method in analyzing the foundational event of rouleaux formation.

The true value of automation extends beyond analytical performance to its transformative impact on laboratory operations and patient care. The workflow analysis presented in Table 1 quantifies a paradigm shift in ESR testing. The manual Westergren method is inefficient and unsafe. The IBP method represents a significant step forward, reducing analysis time by half and improving safety with closed-tube sampling. However, the NIP method offers a truly revolutionary enhancement. Its full integration into a primary hematology analyzer delivers a cascade of benefits that redefine laboratory efficiency. By providing an ESR result in under two minutes from the same K2-EDTA tube used for a complete blood count, it:

- Eliminates Redundant Processes:** It obviates the need for a separate blood draw, a separate sample tube (sodium citrate), and a separate, dedicated analytical workflow;
- Drastically Reduces Turnaround Time:** It shrinks the time-to-result from over an hour to less than two minutes, enabling near-real-time clinical decision-making. This can have a tangible impact on patient flow in emergency departments and allow for more immediate therapeutic adjustments in outpatient clinics;
- Maximizes Staff Efficiency:** It liberates highly skilled laboratory technologists from mundane, manual tasks, allowing their expertise to be redirected to more complex and value-added activities like morphology review or coagulation analysis;
- Enhances Patient Safety and Quality:** By consolidating testing onto a single sample, it minimizes the risk of pre-analytical errors, such as patient identification mix-

ups or incorrect tube draws. The fully automated, closed-tube system provides the highest level of biohazard protection; Enables Advanced Clinical Protocols: The speed and integration of the NIP method allow for the development of sophisticated laboratory protocols, such as automated reflex testing, where an ESR is automatically performed if certain CBC parameters are flagged, providing the clinician with a more complete inflammatory profile without any additional action or delay.²⁰

The strength of this study lies in its rigorous, head-to-head design comparing two distinct automated technologies in a real-world clinical setting and its use of appropriate, advanced statistical models for method comparison. However, certain limitations must be acknowledged. The sample size of 59, while sufficient for the statistical analyses performed, is modest. A larger study would provide greater confidence in the estimates of bias and agreement. Additionally, the study cohort was heavily skewed towards patients with chronic inflammatory diseases. While this provided an excellent spectrum of elevated results, the findings may not be fully generalizable to populations with a higher prevalence of acute infections or specific hematological conditions known to interfere with ESR, such as severe anemia or polycythemia.

5. Conclusion

Both the automated infrared-barrier photometry (IBP) and near-infrared photometry (NIP) methods demonstrate excellent analytical agreement with the Westergren reference method, with no statistically significant systematic bias detected by Passing-Bablok regression analysis. The clinical concordance of both automated methods is exceptionally high, ensuring that their adoption would not lead to significant changes in clinical interpretation or patient management. Both automated technologies offer substantial and quantifiable improvements in laboratory workflow, safety, and efficiency compared to the manual Westergren method. The NIP method, by virtue of its full integration into a primary hematology analyzer, use of a standard K2-EDTA

sample, and near-instantaneous result availability, represents the most advanced and efficient solution for modernizing ESR testing. The findings provide a strong, evidence-based recommendation for clinical laboratories to transition away from the outdated manual Westergren method and adopt these validated automated technologies to enhance the quality and speed of patient care.

6. References

1. Lapić I, Piva E, Spolaore F, Tosato F, Pelloso M, Plebani M. Automated measurement of the erythrocyte sedimentation rate: method validation and comparison. *Clin Chem Lab Med*. 2019; 57(9): 1364–73.
2. Narang V, Grover S, Kang AK, Garg A, Sood N. Comparative analysis of Erythrocyte sedimentation rate measured by automated and manual methods in anaemic patients. *J Lab Physicians*. 2020; 12(4): 239–43.
3. Yang Q, You X, Zhang H, Mwenda K, Wang Y, Huang Y. A new method to predict erythrocyte sedimentation rate with natural geographical factors and location by case-based reasoning: a case study of China. *Chin Geogr Sci*. 2020; 30(1): 157–69.
4. Maki R, Tabe Y, Yamamoto T, Takemura H, Sawada T, Esaki T, et al. Accuracy study of a novel alternate method measuring erythrocyte sedimentation rate for prototype hematology analyzer Celltac α. *Int J Lab Hematol*. 2021; 43(4): 588–96.
5. Orkmez M, Orhan S, Bozdayi MA, Tarakcioglu M. Comparison of the StaRRsed Interliner device with Westergren method in erythrocyte sedimentation rate measurement. *Int J Lab Hematol*. 2021; 43(4): 616–22.
6. Khongwichit S, Saelim M, Na-Songkhla Y, Buncherd H, Nopparatana C, Srinoun K. Method verification of the Caretium XC-A30 automated erythrocyte sedimentation rate analyser for erythrocyte sedimentation rate. *Malays J Med Sci*. 2022; 29(5): 39–47.
7. Prompetchara E, Nowaratsopon S, Wongkamchai S, Srieakpanit J, Ketloy C. Erythrocyte sedimentation rate measurements using MIX-RATE® X20 and VISION A automated analyzers: Method validation and comparison study. *Int J Lab Hematol*. 2022; 44(5): 846–53.
8. Kogovšek R, Meško Brguljan P. M179 Verification of the erythrocyte sedimentation rate measured with two different methods. *Clin Chim Acta*. 2022; 530: S284–5.
9. Higuchi M, Watanabe N. A rapid and accurate method for estimating the erythrocyte sedimentation rate using a hematocrit-corrected optical aggregation index. *PLoS One*. 2022; 17(7): e0270977.
10. Kahar MA. Comparison of alternate erythrocyte sedimentation rate measurement by automated Celltac α+ (MEK 1305) and reference Westergren method. *J Hematol Allied Sci*. 2022; 2(39): 39–45.
11. Brenčić T, Juričić G, Honović L. Performance comparison of Westergren modified SRS 100/II and alternate iSED® method for erythrocyte sedimentation rate. *Int J Lab Hematol*. 2023; 45(1): 29–36.
12. Mujahid A, Choudry JZ, Iqbal H, Zahra SB-E, Latif S, Habib Z. Comparison of erythrocyte sedimentation rate by vision principle and Westergren method. *Pak J Med Health Sci*. 2023; 17(3): 91–3.
13. Itty SS, Priya PV, Anju CK, Sankar S. A comparative study on determination of erythrocyte sedimentation rate by two automated erythrocyte sedimentation rate analysis techniques-Ves Matic Cube 30 and Mixrate X20 in comparison to Modified Manual Westergren method. *J Appl Hematol*. 2023; 14(2): 137–45.
14. Shen Y, Liu D, Wang Y, Cao J, Zhang S, Wen H, et al. Clinical application of a new method for determination of the erythrocyte sedimentation rate using the BC-720

automated hematology analyzer. *Int J Lab Hematol*. 2023; 45(4): 449–59.

15. Cennamo M, Giuliano L, Arrigoni G, Fardone V, Russo R, De Tomasi LM, et al. Method comparison of erythrocyte sedimentation rate automated systems, the VES-MATIC 5 (DIESSE) and test 1 (ALIFAX), with the reference method in routine practice. *J Clin Med*. 2024; 13(3): 847.
16. Miletic M, Barberic M. ISED, CUBE 30 touch and Westergren: Comparison of different methodologies for measuring erythrocyte sedimentation rate (ESR). *Clin Chim Acta*. 2024; 558(118344): 118344.
17. Walle M, Alemayehu E, Tesfaye A, Arkew M, Asmerom H, Agidew MM, et al. Comparison of erythrocyte sedimentation rate measurement between Westergren method and automated method among patients attending Jigjiga University Sheik Hassen Yabare Referral Hospital, Jigjiga, Ethiopia. *Front Med (Lausanne)*. 2024; 11: 1414097.
18. Lorubbio M, Diamanti D, Pieroni C, Gialli E, Pettinari M, Bassi S, et al. Erythrocyte sedimentation rate reference intervals determined via VES-MATIC 5 and CUBE 30 touch with respect to the Westergren method. *Diagnostics (Basel)*. 2025; 15(9).
19. Gori E, Pasquini A, Diamanti D, Carletti C, Marchetti V. Effect of time and storage temperature on canine and feline erythrocyte sedimentation rate. *MethodsX*. 2022; 9(101934): 101934.
20. Shit A, Bora SN. Incorporation of concentration gradient of blood nutrients in erythrocyte sedimentation rate fractional model with non-zero uniform average blood velocity. *Math Methods Appl Sci*. 2024; 47(12): 10334–50.