e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(2); 98-103

Original Research Article

Validation of Automated ESR Method by Capillary Photometry with Conventional Westergren Method

Subinay Datta[1], Biswajit Sarkar[2], Srabani Ghosh[2], Palash Kumar Mandal[3], Payel Das[2], Shreya Halder[2], Mrinal Pal[2]*

[1]Department of Biochemistry, Medical College & Hospital, 88, College Street, Kolkata, West Bengal

[2]Department of Biochemistry, Burdwan Medical College, Near Baburbag Road, Burdwan, West Bengal, India

[3]Department of Pathology, R. G. Kar Medical College, Kolkata

Received: 25-11-2023 / Revised: 28-12-2023 / Accepted: 18-01-2024
Corresponding Author: Mrinal Pal*
Conflict of interest: Nil

Abstract:

Background: Westergren method is routinely used for ESR measurement. However, it has various limiting factors. Alternative method has to be devised to overcome the limitations of the manual Westergren method. These new methods must be properly evaluated before introducing in clinical laboratories.

Methods: A total of 708 randomly collected Ethylene Diamine Tetraacetic Acid (EDTA) samples from patients attending in hospital were assayed parallelly in the recently launched Hematology Analyzer Roller 20 LC and manual Westergren method. Results of these assays were subjected to statistical analysis using a coefficient of correlation and ROC.

Results: The result showed that mean values of different range of blood ESR obtained by Capillary photometry was not significantly differ from gold standard manual Westergren method. To evaluate the correlation of capillary photometric method with reference method Pearson bivariant correlation analysis was performed. Over the whole range of blood ESR values in reference method, the capillary photometric method shows r value ($r = 0.948$, $p < 0.0001$) of blood ESR of same individual. It signifies that values of ESR in capillary photometry of the subjects are significantly correlated with value obtained from reference method. The ROC curves were analyzed and were plotted for the validation of the blood ESR estimation by capillary photometric method. The analysis of the ROC curve illustrated that 0.82 was the area under the curve (AUC) for the method (95% CI, 0.77–0.89, $P < 0.05$).

Conclusion: Hence, it is concluded that in laboratories where the workload is high, automated method of ESR measurement can be conveniently used as a replacement of the standard Westergren method.

Keywords: Automated ESR Methods, Westergren method, Validation, ESR.

Introduction

The erythrocyte sedimentation rate (ESR) is widely used parameter in laboratory as an indicator of inflammation, infection, trauma, or malignant disease. [1] Several methods are used for measurement of ESR such as Westergren method, Wintrobe's method, Zeta sedimentation ratio, and micro-ESR. The most satisfactory method of estimation of ESR was introduced by Westergren in 1921. [2] The Westergren method is recommended for performing the ESR by the International Council for Standardization in Hematology (ICSH). [3,4]

However, ESR measurement by the Westergren method has several limitations such as long analytical time, requirement for large specimen volume, need for diluting specimens, biohazards, and elevation of ESR by anemia. Technical factors such as variation at room temperature, time from specimen collection to test setup, tilting and vibrations, exposure to direct sunlight, improper filling, inconsistent internal boreholes of Westergren tubes, inaccuracy in reading meniscus lines in hazy samples, affect ESR test results, and necessitating the use of alternate test methods.

In 2010 and 2011, ICSH and Clinical and Laboratory Standards Institute (CLSI) released new recommendations. [5,6] They kept the Westergren method as reference procedure and stated that all new technologies, instruments, or methodologies have to be evaluated against the Westergren reference method before being introduced into clinical use. Alternate ESR methods (instruments not based on the Westergren method) use novel approaches such as centrifugation or photometric rheology. [6,7]

The automated Roller 20 LC method is based on the measurement of change in blood impedance after the red cell aggregation-sedimentation phenomenon has occurred. Roller 20 LC works on the principle of photometrical capillary stopped flow kinetic analysis. [8]

Roller 20 LC recreates the physiological body conditions as it is thermostat at 37°C. The inbuilt microcapillary mimics the blood vessel. The blood sample in the capillary is accelerated and immediately stopped in the flow, which is known as stopped flow system. This simulates the blood pressure given by the cardiac muscle, which pumps the blood in the body. Roller 20 LC instrument can measure ESR of 18 samples in 10 minutes with minimal blood volume of 800 µl, whereas the Westergren method takes 60 minutes to interpret the result.

The present study aimed to evaluate the analytic performance of Roller 20 LC for ESR measurement and compare it with the Westergren reference method.

Material and Methods

Study Area: This hospital based cross-sectional study was conducted in the Department of Laboratory Sciences, Lakhotia Diagnostic Services Private Limited (LDSPL), Howrah, West Bengal with the collaboration of Department of Biochemistry of Burdwan Medical College, Burdwan, West Bengal, India.

Ethics Statement: The study was approved and permitted by the institutional ethics committee for care and use of laboratory and started after obtaining the written consent from the concerned ethics committee.

Study samples: The present study was conducted between March 2021 and April 2023. Sample size was calculated at 90% confidence interval, with a margin of error 5% [9] using the formula and 708 random samples were collected from hospitalized and ambulatory patients. Hemolyzed and clotted samples were excluded.

708 samples in total were used for Westergren's (Manual Method) and Capillary photometric (Automated Method). Mean age of the patients was 51.38 ± 20.31 years range from 18 to 79 years, out of total 50.4 % patients were male and 49.9 % were females, 51 % patients from OPD and 49 % were admitted patients shown in Table 1

Table 1: Baseline personal profiles and clinical parameters of the subjects

Study parameters	Data	p value
Age in years	51.38 ± 20.31	
Gender		
Male	357 (50.4)	0.127
Female	351 (49.6)	
Residence		
Urban	355 (50.1)	
Rural	353 (49.9)	0.212
OPD patients	348 (49.1)	
Admitted patients	370 (51.9)	0.115

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables; $p < 0.05$ consider statistically significant

Collection of samples: Peripheral venous 5ml blood was drawn under aseptic precautions in disposable blood collection tubes having potassium EDTA as the anticoagulant. Samples were stored at room temperature and analyzed within 4 h of collection both by manual Westergren method and Capillary photometric method.

ESR measurement by Capillary photometric method: Automated mixing of EDTA sample tube was performed for 2 min to ensure complete disaggregation of erythrocyte before analyzing the sample in the automated analyzer. Samples were assayed as per the operator's manual.[10]

Manual measurement of ESR by Westergren method: Westergren Method is a tube with an open end and a length of 30 centimetres and a diameter of 2.55 millimetres is referred to as a Westergren tube. At the top, it contains the number 0, and at the bottom, it has a graduation of 170 millimetres. In a test tube, 4 volumes of venous blood were anticoagulated with 1 volume of either trisodium citrate or EDTA at a concentration of 3.2 percent. The Westergren tube is then filled with this diluted blood to 0 marks with suction applied by finger over the upper end of the tube. The tube was allowed to stand for 1 hour and results were interpreted exactly after an hour in mm/hour.

Quality check of automated analyzer using control materials: Alifax Latex control (Lot No. 2404D) from R & D, Design and manufacturing Dept, via Merano, 30-33045 Nimis (UD) - Italy were assayed once a day at the start of the shift to ensure the appropriate functioning of the ROLLER20LC analyzer.

Precision check of ROLLER20LCanalyzer using hematology controls and patient: Between-run precision was performed with normal and abnormal

with same lot number controls and analyzed once a day for 40 consecutive days. Results of 40 consecutive assays of controls were used to determine precision for the ROLLER20LC. Moreover, data of nine random patient samples (each assayed 11 times on same day) on the hematology analyzer were also used to determine precision (repeatability).

Statistical Analysis: The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows. Comparison of ESR value of two methods of blood ESR estimation was performed using Unpaired t test. Pearson bivariant correlation helps to evaluate the any correlation of capillary photometric method with reference method. Then validation of the blood ESR estimation by capillary photometric method was analyzed by Receiver Operating Characteristic (ROC) Curve Analysis.

Result

Comparison of ESR value of two methods of blood ESR estimation – Unpaired t test: In Table 2, mean values of different range of blood ESR obtained by Capillary photometry was not significantly differ from gold standard manual Westergren method.

Table 2: Comparison of ESR value of two methods of blood ESR estimation.

ESR range	Total samples	Method		Mean difference	p value
		Manual Westergren (mm/hour)	Capillary photometry (mm/hour)		
1–20 mm/h	347	10.94 ± 4.91	8.26 ± 2.07	2.68	0.142
21–60 mm/h	226	37.69 ± 9.84	32.71 ± 8.28	4.98	0.084
>60mm/h	135	82.11 ± 14.06	77.38 ± 9.58	4.73	0.091

Values are mean ± SD

Validation of the blood ESR estimation by capillary photometric method- Receiver Operating Characteristic (ROC) Curve Analysis: The ROC curves were analyzed and were plotted as shown in Figure 1 for the validation of the blood ESR estimation by capillary photometric method. The analysis of the ROC curve illustrated that 0.82 was the area under the curve (AUC) for the method (95% CI, 0.77–0.89, P < 0.05).

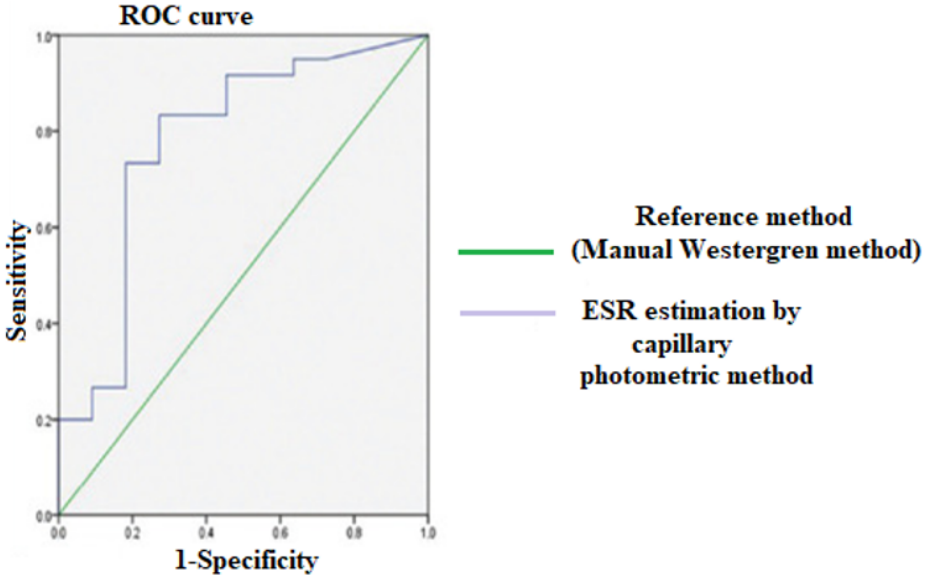

Figure 1: Receiver Operative Characteristic (ROC) curves of reference method (manual Westergren method) and capillary photometric methods of blood ESR (AUC:0.82)

Correlation of capillary photometric method with reference method - Pearson bivariant correlation: Pearson bivariant correlation analysis is performed to evaluate the correlation of capillary photometric method with reference method as shown in Table 3. Over the whole range of blood ESR values in reference method and the capillary photometric method shows r value (r = 0.948, p < 0.0001) of blood ESR of same individual. It signifies that values of ESR in capillary photometry of the subjects is significantly correlated with value obtained from reference method (Figure 2)

Table 3: Bivariant Correlation of capillary photometric method with reference method

ESR ranges	Total samples	r (95% confidence interval)	Intercept (95% Confidence interval)	Slope (95% Confidence interval)	Mean bias (95% Confidence interval)	p value
Low range (1–20 mm)	347	0.667 (0.575 – 0.736)	−4.08 (−6.27–−3.15)	0.82 (0.64–0.98)	−5.51 (−6.32 to −4.61)	<0.0001
Middle range (21–60 mm)	226	0.657 (0.551 – 0.743)	−22.05 (−30.25–−14.65)	1.42 (1.17–1.73)	−7.17 (−9.31 to −4.82)	<0.0001
Upper range (>60 mm)	135	0.637 (0.438 – 0.772)	−67.8 (−114.0–42.38)	1.75 (1.38–2.21)	−7.37 (−13.74 to −0.78)	<0.0001
All ranges	708	0.909 (0.889 – 0.926)	−6.16 (−7.0–−5.0)	0.96 (0.90–1.00)	−6.43 (−7.71to −5.14)	<0.0001

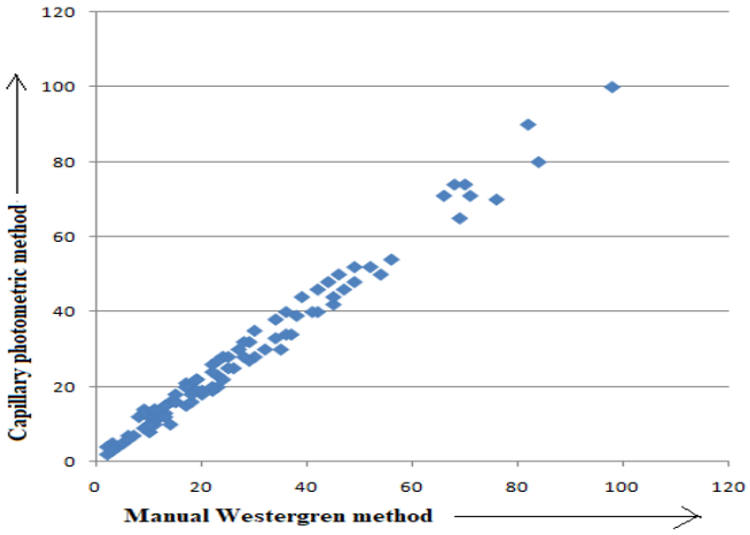

Figure 2: Scatter diagram of correlation of capillary photometric method with reference method

Results for precision studies using commercial control and patient samples: Inter-run precision analysis of commercial control samples and patient samples once a day for 40 days yielded inter-run CV of 1.9% for the abnormal range control and 2.1% for the normal range control.

Intrarun precision: Nine patient samples were assayed 11 times for assessing intrarun precision. Intrarun CV% obtained ranged from 0% to 5%

Discussion

ESR is commonly used as an indicator of inflammation and infection, although it is not a specific test. But it is a quite simple and inexpensive parameter used to assess patients with acute or chronic inflammatory conditions. [11,12]

ESR estimation helps to serve as a useful aid in the diagnosis of different clinical conditions, and has prognostic role in neoplastic diseases and coronary heart disease. [13-16] Westergren method is the commonly used method for estimation of ESR. But many internal and external factors can influence the ESR results obtained by the Westergren method. Moreover, the modified Westergren method is laborious, cumbersome, needs large volume of blood, long analysis time (>1 h), and carries the risk of infection. For practical reasons, the Westergren method is diminishingly used for ESR determination and majority of laboratories have started using alternate or modified methods.

ROLLER20LC analyzer being an automated closed system based on capillary photometric method that offers many advantages such as ease of performance, safety, savings on consumables, use of EDTA sample, need for less sample volume, and less turnaround time. The analyzer additionally gives the results along with complete blood count to its utility in the hematology laboratories.

In recognition of the need for a standardization of the measurement of ESR, the ICSH has proposed a protocol for the evaluation of alternative methodologies against the reference method has also been

proposed.[11,12] The new technologies must be tested over a range of ESR values of 1–120 mm. In this comparison, 95% of the difference should be 5 mm or less, with larger differences associated with higher ESR values. The statistical methods recommended for ESR evaluations are the coefficient of correlation, and the Receiver Operative Characteristic (ROC) curves.

We carried out this study to look for correlation of automated method (capillary photometric method) with Westergren method. We found strong positive correlation of both the methods with Westergren method with highly significant p value of <0.0001. We also calculated Coefficient of variance for the methods and it was found that the automated method was well correlated with old and gold standard Westergren method Other studies also confirmed the excellent correlation.[11,13,14]

Our findings are entirely consistent with the previous research which showed the positive correlation between conventional method and automated vacuette as calculated by Pearson correlation coefficient and statistically significant p-value < 0.001 contrary to our value which came out to be statistically insignificant > 0.05. [11,13,14]

Our results also coincide with the study conducted at faculty of Allied Health Sciences Kuwait. [15,17] The study also showed that the efficacy of conventional method is very much similar to the automated SEDI system but there was also a little discrepancy noticed on Bland and Altman statistical method.

Furthermore, our results are completely opposing the results found in anemic patients in Tertiary Referral Institute of North India [8,18] where there was clearly shown in results that automated method such as Roller 20 LC can safely replace the Westergren method in the heavy work load environment. So from our study we can correlate our findings with the previous studies where there is a strong correlation between the two method but Westergren method is found to be more efficient, accurate and authentic as shown by coefficient of variance in our study which proved that manual method in spite of the more time consuming and carrying a few by hazard risk has proven to be more reliable as compared to automated ISED (ALCOR) method.

In another study, the investigator used automated instrument MONITOR 100 from Electa Laboratory Italy and divided ESR of 200 patients having HCT between 30 to 36% into 0–25 mm/hr (n = 79) and > 25 mm/hr (n = 121) ranges. They found mean difference of just–7.7 and 95% limit of agreement between –18.9 to 3.5 for ESR values less than 25 mm/hour compared with mean difference of 13.4 and 95% limits of agreement between –57.3 to 30.5 for high values. [19] Similarly, on analyzing SEDI system (Becton Dickinson, MeylanCedex, France), in other study it showed low agreement between the automated and Westergren methods at the higher ESR values as compared with normal ranges. They also reported that for samples with ESR readings > 25 mm/hr (n = 81), the mean of difference (–21.4) and the 95% limits of agreement (–45.2 and 2.26) were markedly different from the corresponding values (–3.9,–13.5 and 5.7, respectively) for samples with ESR values < 25 mm/h (n = 69). [20]

Conclusion

Hence, it is concluded that in laboratories where the workload is high, automated method of ESR measurement can be conveniently used as a replacement of the standard Westergren method.

Acknowledgement: We, the authors are thankful for the contribution of Late (Mr) Nishikanta Pramanick.

References

1. Plebani M, Piva E. Erythrocyte sedimentation rate: use of fresh blood for quality control. Am J Clin Pathol 2002;117(4):621–626.
2. Westergren A. Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Med Scand. 1921; 54:247–282.
3. Bull BS, Brecher G. An evaluation of the relative merits of the Wintrobe and Westergren sedimentation methods, including hematocrit correction. Am J Clin Pathol 1974;62(4):502–510.
4. Moseley DL, Bull BS. A comparison of the Wintrobe, the Westergren and the ZSR erythrocyte sedimentation rate (ESR) methods to a candidate reference method. Clin Lab Haematol 1982;4(2):169–178.
5. Clinical Laboratory Standards Institute (CLSI), Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard (5th ed., H2–A5). Villanova, PA: CLSI; 2011.
6. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hematol 2017;39(5):448–457.
7. Aytekin M. The current use and the evolution of erythrocyte sedimentation rate measurement. Middle Black Sea J Health Sci 2018; 4:17-23.
8. Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S; International Council for Standardization in Haematology. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol 2011;33(2):125–132.
9. Available from: https:// www.calculator.net/ Sample-Size-Calculator.html
10. Operator's Manual of ROLLER20LC Automated Hematology and ESR Analyzer, Italy. http://dx.doi.org/10.1515/cclm-2019-0204

11. Alexy T, Pais E, and Meiselman HJ. A rapid method to estimate Westergren sedimentation rates. Review of scientific instruments 2009; 80: 096101-3.
12. International Council for Standardization in Haematology, J. Clin. Pathol 1993; 46: 198.
13. J. G. Jones and B. L. Hazleman. ESR in polymyalgia rheumatica and giant cell arteritis Ann. Rheum. Dis 1983; 42: 702-03.
14. Bird HA, Esselinck W, Dixon AS, Mowat AG, Wood PHN: An evaluation of criteria for polymyalgia rheumatica. Ann Rheum Dis 1979; 38: 434–439.
15. R. C. Brooks and S. R. McGee, Arch. Diagnostic dilemmas in polymyalgia rheumatica Arch Intern Med 1997;157(2):162-8.
16. J. Danesh, R. Collins, R. Peto, and G. D. Lowe Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease Eur Heart J. 2000;21(7):515-20.
17. Vikram Narang, Sumit Grover, Amandeep Kaur Kang, Avantika Garg, Neena Sood, comparative analysis of Erythrocyte sedimentation rate measured by Automated and Manual methods in anaemic patients. J. Lab Physicians, 2020 Dec; 12(4): 239-243.
18. Fiorucci G, Camogliano L and Massacane R. comparison of two automated systems for the measurement of Erythrocyte Sedimentation Rate: Ves-Matic and Test 1. Biochemica Clinica 2000; 24(3): 175-179.
19. Subramanian A, Rangarajan K, Pandey RM, Gandhi JS, Sharma V, Bhoi SK. Evaluation of an automated erythrocyte sedimentation rate analyzer as compared to the Westergren manual method in measurement of erythrocyte sedimentation rate. Indian J PatholMicrobiol 2011;54(1):70–74.
20. AlFadhli SM, Al-Awadhi AM. Comparison of erythrocyte sedimentation rate measurement by the automated SEDIsystem and conventional Westergren method using the Bland and Altman statistical method. Med PrincPract 2005; 14(4): 241–244.