

Erythrocyte Sedimentation Rate measured by Automated Analysers and Manual Westergren's Method – A Comparative Study**Karthika V.¹, Shyamala Gowri M.², D. Arunthamizhpraba³, Soundarrajan T.⁴, K. Raghul⁵**¹Senior Resident, Department of Pathology, Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur, Perambalur, Tamil Nadu, India²Senior Resident, Aarupadai Veedu Medical College and Hospital, Vinayaka Missions Research Foundation, (Deemed to be University), Puducherry, India³Senior Resident, Department of Pathology, Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur, Perambalur, Tamil Nadu, India⁴Assistant Professor, Department of Forensic Medicine & Toxicology, Dhanalakshmi Srinivasan Medical College & Hospital, Perambalur, Siruvachur, Tamil Nadu, India⁵Assistant Professor, Department of Forensic Medicine & Toxicology, Dhanalakshmi Srinivasan Medical College & Hospital, Perambalur, Siruvachur, Tamil Nadu, India

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Corresponding author: Dr. Karthika V.

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Abstract**Background:** Erythrocyte Sedimentation Rate (ESR) determined by Westergren's method is used in diagnosis and monitoring of inflammatory activities; however, it has many limitations which include inherent and technical factors. Alternate methods have been introduced to overcome the limitations of the manual method. These new methods must be properly evaluated before introducing in clinical laboratories.**Materials and Methods:** A total of 436 randomly collected blood samples were assayed simultaneously by the standard Westergren's method and two automated methods using Roller 20LC and Celltac $\alpha+$ MEK-1305.**Results:** Results of these assays were subjected to statistical analysis using a Spearman rank coefficient of correlation, the Bland-Altman statistical methods and Passing-Bablok regression method. The present study on comparison of manual Westergren's with Roller 20LC and Celltac $\alpha+$ MEK-1305 revealed that the Spearman rank correlation coefficient ' ρ ' of 0.781 (95% confidence interval [CI] 0.742 to 0.815, $P < 0.0001$) and 0.774 (95% confidence interval [CI] 0.733 to 0.809, $P < 0.0001$), mean bias of -2.43 and -8.25 respectively. The limits of agreement are -35.8 to 30.9 and -53.8 to 37.3 between Roller 20LC and Celltac $\alpha+$ MEK-1305 with the reference Westergren's method. With its added benefits, automated Roller 20LC followed by Celltac $\alpha+$ MEK-1305 are legitimate replacement for the reference ESR method in clinical laboratories due to its good correlation with the reference Westergren's method, acceptable bias and limits of agreement.**Keywords:** Erythrocyte Sedimentation Rate (ESR), Roller 20LC, Westergren's, Celltac $\alpha+$, Sedimatic.**DOI:** 10.25258/ijcpr.18.4.158This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

In clinical practice, the Erythrocyte Sedimentation Rate (ESR) is frequently utilized as a marker of inflammation, infection, trauma, or malignancy disease [1,2]. Westergren's and Fahraeus developed an extremely effective procedure in 1921, which is how the test got its name [3]. The preferred method for measuring ESR is Westergren's method, according to the recommendation of the International Council for Standardization in Haematology (ICSH) [4,5]. Normal value of ESR in adults ranges from 2 to 20mm at the end of one hour [6]. Many factors affect ESR value such as red blood cells to plasma,

cellular factors like cell size, cell surface and their intrinsic capability to aggregate and sediment [7]. Zeta potential plays an important role in measuring ESR. Increased in rouleaux formation is due to increased plasma proteins such as haptoglobin, ceruloplasmin, alpha1- acid glycoprotein, alpha 1- antitrypsin and C-reactive protein (CRP) [8,9]. ESR measured by the manual Westergren's method is affected by many factors such as room temperature and length and angle of placement of the tube [10]. And it is also affected by haematocrit, which can be corrected by Fabry's formula (Westergren's ESR X 15/55-HCT) [11]. Recently many new automated

techniques for measuring ESR have been developed and introduced in clinical laboratories, to overcome the confounding factors. They also provide many advantages like safety of operators, reducing biohazards risks, quicker results, speedy processing time, and performance of other haematological tests (erythrocyte, leucocyte, Mentzer index and RDWI) in a single sample.

In 2010 and 2011, the International Council for Standardization in Haematology (ICSH) and Clinical & Laboratory Standards Institutes (CLSI) released new recommendations; they kept the Westergren's method as reference procedure and stated that all new technologies, instruments or methodologies have to be evaluated against the Westergren's reference method before being introduced into the clinical use. [12,13] Celltac α + MEK-1305 is an automated machine for measuring CBC and ESR. The advantage with this method is that it can give the ESR readings in two minutes and it can also measure CBC + WBC 3 part differential + ESR of approximately 20 samples / hour in normal mode with the minimal blood volume of 80 μ L and it is using EDTA anticoagulated blood sample. ESR is calculated from sylectogram, HCT (haematocrit) and MCV (Mean corpuscular volume). [14]

The automated Roller 20LC method is based on the measurement of change in blood impedance after the red cell aggregation –sedimentation phenomenon has occurred. Roller 20LC works on the principle of photometrical capillary stopped flow kinetic analysis and it is using EDTA anticoagulated blood sample. The inbuilt micro capillary mimics the blood vessels. The stopped flow system is the result of the blood sample in the capillary being accelerated and then abruptly stopped in the flow. Roller 20LC instrument can measure ESR of 18 samples in 10 minutes with minimal blood volume of 800 μ L, whereas Westergren's method takes 60 minutes to interpret the results. [6]

The present study aimed to compare the accuracy of ESR values measured by Celltac α + MEK-1305 and Roller 20LC, two automated ESR analysers with the gold standard manual Westergren's method.

Materials and Methods

This study was carried out over a six-month period at the Haematology Laboratory, IGMC & RI, Puducherry, after IRC and IEC approval.

ESR assessments were performed on 436 consecutive EDTA blood samples collected from both male and female patients over the age of 18. Blood samples that were diluted or clotted, as well as those with incorrect labelling of details, were removed from the study. Samples were maintained

at room temperature and analyzed within 4 hours of collection using the usual Westergren's method and two automated methods, Celltac α + MEK-1305 and Roller 20LC. The samples were gathered anonymously, with no identifying information recorded for the study. Data was entered into an MS Excel sheet and analyzed using SPSS software version 20. The Celltac α + MEK-1305 and Roller 20LC techniques were evaluated according to Bland-Altman guidelines. Manual Westergren's approach is regarded the gold standard. The mean ESR value for all three methods was shown against the difference between Westergren's and automated procedures (Celltac α + MEK-1305 & Roller 20LC). The 95% limit of agreement was estimated as $d \pm 1.96$ SD, where d represents the mean difference between two measurements and SD is the standard deviation of differences. P-values < 0.05 are considered significant. The correlation coefficient was calculated between automated analysers and the manual Westergren's technique. The value of " ρ " was taken as 0.00 to 0.20-extremely weak; 0.21 to 0.40-weak; 0.41 to 0.60-moderate; 0.61 to 0.80-strong; and 0.81 to 1.00-very strong. Pearson correlation (or Spearman correlation) was utilized depending on the data's normality. Linear regression analysis was performed using the Passing-Bablok method, with Westergren's method as the dependent variable and one of the automated analysers as the independent variable. R-squared values were calculated for both equations. Westergren's approach ESR data were split into three groups: 1 to 20mm/hour (low range); 21 to 60mm/hour (middle range); and >60 mm/hour (upper range) [14]. The aforementioned statistical analysis was repeated for these three subgroups.

The calculations were performed using "MedCalc® Statistical Software" version 22.023 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2024).

Results

A total of 436 random samples were tested simultaneously using the standard Westergren's method and two automated methods using Celltac α + MEK-1305 and Roller 20LC. The results are as follows. The 436 ESR values were divided into three categories: 1-20mm/h, 21-60mm/h, and >60 mm/h. Westergren's approach yielded ESR readings (mean 29.74 \pm SD 23.89) for the entire range (1-190mm/h), which were not substantially different from Roller 20LC (mean 32.17 \pm SD 27.32) but only slightly different from Celltac α + MEK-1305 (mean 37.99 \pm SD 35.83). Table 1 displays the results of ESR measurements for 436 samples using Westergren's technique, Roller 20LC, and Celltac α + MEK-1305 in various ranges, as well as statistical calculations (mean, standard deviation, and standard error).

Method Comparison Results

ESR measurements in 436 samples yielded a median of 30 mm/h using Westergren's technique, 26 mm/h with Roller20LC, and 22 mm/h with Celltac α+ MEK-1305. The Spearman rank correlation coefficient (ρ) with Roller 20 LC was 0.781 (95% confidence interval [CI] 0.742 to 0.815, P<0.0001), and with Celltac α+ MEK-1305 was 0.774 (95% confidence interval [CI] 0.733 to 0.809, P<0.0001). The bias between the Westergren's method and Roller20LC was -2.43

mm/h, whereas the bias between the Westergren's method and Celltac α+ MEK-1305 was -8.25 mm/h. Zero is the predicted value if the two methods measure the same sample identically. The Westergren's technique had limits of agreement with Roller20LC (-35.8 to 30.9), and Celltac α+ MEK-1305 (-53.8 to 37.3). Figure 1a and 1b, Table 2 displays the technique comparison results (Spearman rank correlation coefficient ρ, intercept, slope, and mean bias) for ESR measurement using Westergren's method, Roller20LC, and Celltac α+ MEK-1305 in three groups.

Table 1: ESR measurements Comparison

ESR Range	N	Method	Mean	SD	SEM
1-20	195	Manual	9.17	5.43	0.39
		Roller 20LC	13.81	13.2	0.94
		Celltac α+	14.93	13.09	0.94
21-60	192	Manual	38.09	9.87	0.71
		Roller 20LC	38.97	20.1	1.45
		Celltac α+	46.11	28.84	2.08
>60	49	Manual	78.88	14.23	2.03
		Roller 20LC	78.57	26.47	3.78
		Celltac α+	97.96	39.21	5.6
Complete (1-190)	436	Manual	29.74	23.89	1.14
		Roller 20LC	32.17	27.32	1.31
		Celltac α+	37.99	35.83	1.72

*SD- Standard Deviation, SEM- Standard Error of mean

Table 2: Results of comparing ESR measurement

ESR Range	N	Method	P(95% confidence interval)	Intercept(95% confidence interval)	Slope(95% confidence interval)	Mean Bias(95% confidence interval)
1-20	195	Roller 20LC	0.424(0.302 to 0.533)	-6.0(-10 to -3.875)	2.0(1.688 to 2.5)	-4.64(-6.431 to -2.841)
1-20	195	Celltac α+	0.444(0.324 to 0.550)	-5.46(-10.02 to -2.8)	2.09(1.733 to 3.0)	-5.75(-7.52 to -3.993)
21-60	192	Roller 20LC	0.383(0.255 to 0.497)	-55.59(-75 to -38.5)	2.55(2.10 to 3.13)	-0.88(-3.528 to 1.768)
21-60	192	Celltac α+	0.382(0.254 to 0.497)	-96.0(-143.5 to -65.75)	3.8(2.95 to 5.10)	-8.02(-11.845 to -4.186)
>60	49	Roller 20LC	0.320(0.043 to 0.552)	-162.4(-524.0 to -79.0)	3.08(2.0 to 7.8)	0.31(-6.461 to 7.074)
>60	49	Celltac α+	0.308(0.0295 to 0.542)	-246.8(-629.0 to -123.5)	4.47(2.85 to 9.4)	-19.08(-29.175 to -8.989)
Complete	436	Roller 20LC	0.781(0.742 to 0.815)	-1.4(-2.4 to 0.33)	1.133(1.067 to 1.2)	-2.43(-4.028 to -0.825)
Complete	436	Celltac α+	0.774(0.733 to 0.809)	-2.0(-3.9 to -1.04)	1.30(1.208 to 1.40)	-8.25(-10.43 to -6.06)

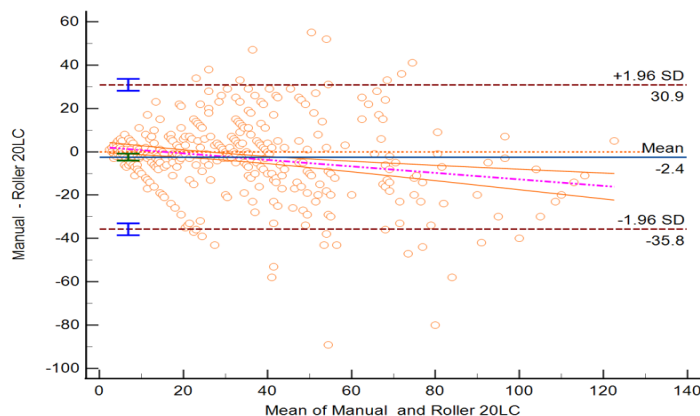


Figure 1a: Bland - Altman Plot comparing the difference between ESR values acquired using Westergren's method and those obtained using the Roller 20LC (Y-axis) versus the mean of ESR values (manual Westergren's + Roller 20LC) (X-axis). The dotted lines represent limits of agreement (-35.8 to 30.9), and the bias is -2.4.

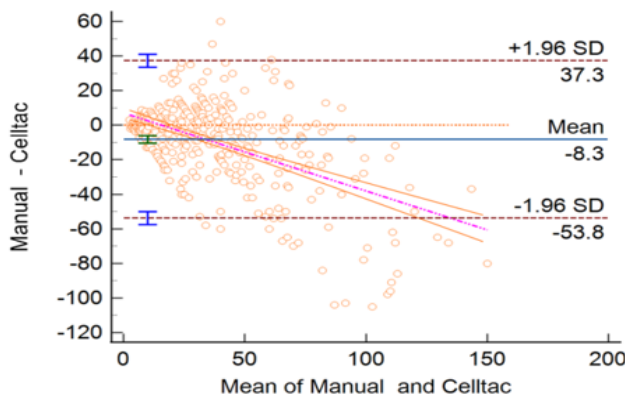


Figure 1b: Bland - Altman plot comparing the difference between ESR values acquired using Westergren's method and those obtained using the Celltac α + MEK-1305 (Y-axis) versus the mean of ESR values (manual Westergren's + Celltac α + MEK-1305) (X-axis). The dotted lines represent limits of agreement (-53.8 to 37.3), and the bias is -8.3.

Discussion

ESR determined using manual Westergren's method is the gold standard procedure¹⁵. Due to its inexpensive cost, simplicity of use and familiarity with assessing acute phase inflammation response, ESR is performed in many laboratories. ESR remains helpful in the precise identification of certain disorders such Polymyalgia rheumatica, temporal arteritis and Rheumatoid arthritis [12].

Despite the existence of numerous alternative approaches, Westergren's method for determining ESR remains the accepted method. However, the use of automated analysers limits the number of physiological and technical factors that affect the ESR determination by Westergren's approach. [16,17] In the 1990s, a number of new automated systems have been created and their performance has been assessed against the gold standard Westergren's method, as well as against each other. Some of them are includes Ves-matic 60, Sediscan, Sedimatic , Roller 20 LC, Test 1 automated ESR analyser, Celltac α + MEK-1305 and others.

Although these automated techniques offers more benefits in terms of reduced biohazard risks, speedy processing time, and quicker results, it is essential to validate those equipment against the standard Westergren's method to enable routine use and to replace the standard ESR method in any hospital. [1] In the present study, the results obtained with the automated analysers were compared with the gold standard manual Westergren's method using the agreement analysis of Bland and Altman. Statistical analysis such as linear regression and correlation, though used commonly in the evaluation of new equipment, is usually not considered to be appropriate for validating equipment. Agreement analysis is more sensitive method than the correlation coefficient for comparison between the two methods.¹⁸ Bland and Altman analysis between the Westergren's method and Roller20LC demonstrated an acceptable overall bias of -2.4 and limits of agreement -35.8 to 30.9 for 436 samples. The bias in this study was positively increasing for higher values of ESR; bias of -4.6 in low range (1-20mm/h), bias of -0.8 in

middle range (21-60mm/h), and bias of 0.30 in upper range (>60mm/h) which means that the Roller 20LC was overestimate the manual Westergren's readings for ESR values on the higher range. Bland and Altman analysis between the Westergren's method and Celltac α+ MEK-1305 demonstrated an acceptable overall bias of -8.2 and limits of agreement -53.8 to 37.3 for 436 samples. The bias in this study was negatively increasing for higher values of ESR; bias of -5.75 in low range (1-20mm/h), bias of -8.01 in middle range (21-60mm/h), and bias of -19.08 in upper range (>60mm/h) which means that the Celltac α+

MEK-1305 was underestimate the manual Westergren's readings for ESR values on the higher range. Linear regression analysis of Roller 20LC and Celltac α+ MEK-1305 according to Passing-Bablok $\{(y=-1.400 + 1.333 x)$ and $(y = -2.000 + 1.300 x)$ } respectively, indicated high concordance between results obtained by the two automated analysers and the Westergren's method [Figure 2a and 2b].The Spearman rank correlation coefficient ρ for Roller 20 LC and Celltac α+ MEK-1305 was 0.781 and 0.774 respectively, indicating a good correlation between the two automated analysers and the Westergren's method.

Table 3: Data from several studies comparing Westergren's method to other automated methods for determining ESR performance evaluation

Author	Equipment used	Sample size	Linear regression	Passing-Bablok	Bland-Altman		
					Bias	Limits of agreement	95% CI
Lapic et al. [19]	Ves-Matic cube 200	448	$\rho = 0.852$	$y = 0.98x + 1.4$	-0.3	-33.7to33	-1.9to1.2
Maki et al. [20]	Celltac α+ MEK-1305	271	$r = 0.945$	$y = 1.026x + 0.46$	-	-	-
Kahar MA [14]	Celltac α+ MEK-1305	350	$r = 0.905$	$y = 0.962x - 6.61$	-6.43	-30.4to17.5	-7.71to-5.14
Perovic et al. [21]	Ves-Matic cube 200	250	$\rho = 0.946$	-	-0.5	-13.0to12.9	-0.37to1.32
Sezer et al. [22]	Ves-Matic cube 200	101	$\rho = 0.82$	$y = 1.15x - 2.59$	-0.7	-32.6to31.2	-
Bogdaycioglu et al. [23]	iSED	136	$r = 0.76$	$y = 0.74x + 0.07$	13	-35.7to61.6	
	Ves-Matic cube 200	136	$r = 0.84$	$y = 0.92x + 1.25$	1.4	-34.4to37.2	
Schapkaitz et al. [24]	iSED	120	$r = 0.88$	-	7.9	-	-5.87to10.13
Kim et al. [25]	Test 1	195	-	$y = 0.73x - 8.22$	-26.7	-84.0to30.7	-30.8to-22.5
Present study	Roller 20LC	436	$\rho = 0.781$	$y = 1.333x - 1.4$	-2.4	-35.8to30.9	
	Celltac α+ MEK-1305	436	$\rho = 0.774$	$y = 1.300x - 2.0$	-8.2	-53.8to37.3	

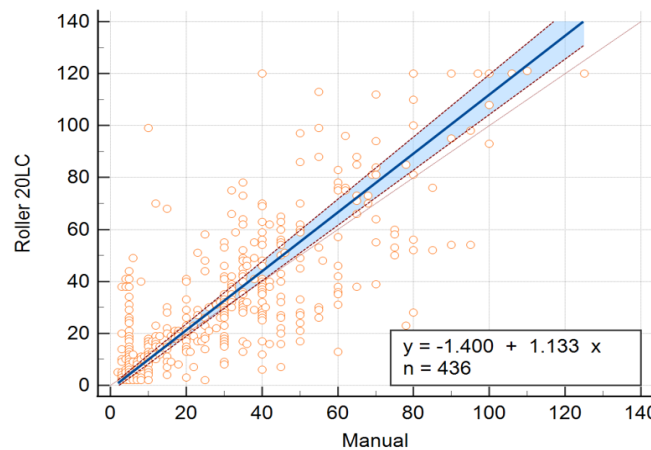


Figure 2a: Passing-Bablok analysis for comparison of Roller 20LC and the Westergren's method: $y = -1.400 + 1.133x$.

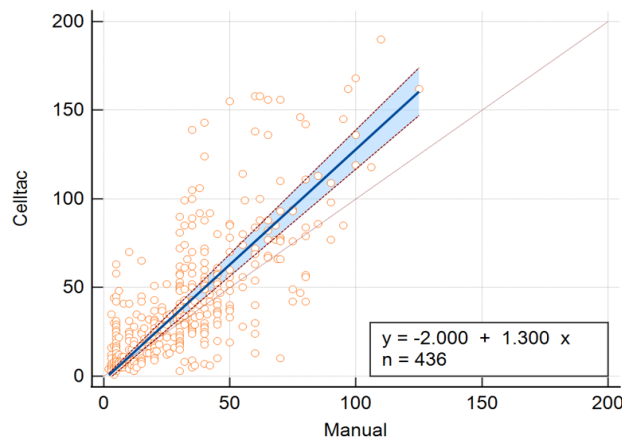


Figure 2b: Passing-Bablok analysis for comparison of Celltac $\alpha+$ MEK-1305 and the Westergren's method: $y = -2.000 + 1.300x$.

The linear regression values of Roller 20LC and Celltac $\alpha+$ MEK-1305 in our investigation (0.7) are found to be comparable to those of the iSED automated analyzer study conducted by Bogdaycioglu et al. [23]. The linear regression value in additional research by Lopic et al [19], Sezer et al [22] and Schapkaitz et al [24] is 0.8 and Maki et al [20], Kahar MA [14] & Perovic et al [21] is 0.9. This clearly shows that all of the research employing various automated analyzers have excellent correlations with the gold standard Westergren's approach. The above table depicts data from several studies comparing Westergren's method to other automated methods for determining ESR performance evaluation.

Conclusion:

The Roller 20LC and Celltac $\alpha+$ MEK-1305 automated analysers showed good correlation with the reference Westergren's method and an acceptable bias of overall range of ESR, exhibiting satisfactory concordance of ESR results between the two automated analysers and the reference Westergren's method.

Automated analysers have advantages with reduced sample volume, ease of performance, reduced biohazard and reliability, making it a valid substitute for reference Westergren's method for ESR determination with periodic quality control. But discrepancies in the ESR results were observed with higher ESR values.

However, it is not evident for normal ESR values. Thus the Roller 20LC automated system tended to overestimate whereas Celltac $\alpha+$ MEK-1305 automated system underestimated the manual readings for ESR values on the higher range which is clinically unacceptable.

Hence it is advisable to confirm the higher ESR values of automated machines with manual Westergren's method, as it is a gold standard.

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