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Comparative study of erythrocyte sedimentation rate measurement between Celltac Alpha+ (MEK-1305) ESR Analyzer and standard Westergren method

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ABSTRACT

Background: The Westergren method, a gold standard method for erythrocyte sedimentation rate (ESR) measurement, is one of the screening tests for routine clinical hematology that can be used as an inflammation marker. Since the results can be altered by numerous factors, new methodologies have been established to manage the possible pitfalls.

Objective: This study compared erythrocyte sedimentation rate measurements obtained by the Celltac Alpha+ (MEK-1305) Automated Hematology and ESR analyzer with those obtained by the standard Westergren method.

Materials and methods: The Celltac Alpha+ (MEK-1305) Automated Hematology and ESR analyzer was used to assess the performance of ESR measurement compared to the standard Westergren method. A total of 220 random EDTA whole blood samples from patients were included and analyzed in parallel using the automated analyzer and the standard Westergren method.

Results: Spearman's rank correlation coefficient (ρ = 0.916) obtained a good correlation between the two methods. In addition, the precision was good and acceptable according to the criteria, with a %CV of less than 10% in both intra-run and inter-run precision. There was no contamination between blood samples from using a single capillary probe. Moreover, Bland-Altman mean difference plot also indicated a good agreement, with a mean bias of -1.4 (95% CI: -0.8 to 2.1), an upper LOA of 7.6 (95% CI: 6.6 to 8.7) and a lower LOA = -10.5 (95% CI: -11.6 to -9.5).

Conclusion: This comparative study indicated that the Celltac Alpha+ (MEK-1305) Automated Hematology and ESR analyzer is applicable within clinical routine practice along with proper measurement regulation. Even though the principles between these two methods are completely different, an acceptable interrelation was obtained due to the calibration process by Nihon Kohden corporation.

Introduction

Westergren method, a gold standard method for erythrocyte sedimentation rate (ESR) measurement, is one of the common routine hematology tests that can be used as an inflammatory and infection marker.[1] In recent years, high-sensitivity C-reactive protein (hs-CRP), rheumatoid factor (RF), and interleukin-6 (IL-6) become more noticeable within clinical laboratory fields due to their higher specificity. However, physicians still request the measurement of an ESR. Formerly, ESR was integrated with other diagnostic criteria for many conditions such as rheumatoid diseases, autoimmune diseases, infections,

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and tumors.[2] The authentic measurement of an erythrocyte sedimentation rate, Westergren method, was performed by determining the rate of red blood cell sedimentation by observing the level to which the cells fall in a given time interval. Conventionally, a whole blood sample mixed with a particular amount of diluent is aspirated into a specific vertical tube, straightly stand for 1 hour, and recorded the plasma length in millimeters/hour (mm./hr.).[3] However, numerous variables can change the pace at which erythrocytes settle out. Various factors, both physiological and pathological, as well as laboratory conditions, can affect ESR levels. Menstruation, pregnancy, malignancies, incorrect anticoagulant use, and elevated temperatures are among the circumstances that may elevate ESR values. In contrast, lower ESR levels may be observed in males, as well as in cases involving hemolyzed blood samples, polycythemia vera, and plasma hyper-viscosity.[4-7]

The standard Westergren method is not advisable for routine laboratory testing because of its long assay time, complicated procedure, and high risk of contamination. Over decades, automation has been continuously invented and developed for application in clinical laboratory practice and to complete its main purposes, including workload reduction, turnaround time shortening, worker safety improvement, and human error lessening. Celltac Alpha+ (MEK-1305) automated hematology and ESR analyzer can measure 20 hematological parameters, including the ESR. The automated hematology analyzer uses optical measurement of the rouleaux formation and aggregation of red blood cells that occur in the initial phase of the sedimentation phenomenon to measure ESR quickly. Rouleaux formation and aggregation of red blood cells are not equivalent to the sedimentation rate. Still, by utilizing the hematocrit (Hct) and mean corpuscular volume (MCV) measurement results, which are closely related to ESR (1-hour value), the analyzer can achieve an ESR value in a shorter amount of time that has a high correlation with the reference method. In the ESR measuring unit, light from an LED is emitted into the agitated blood, and the light that passes through is continuously measured by a light-receiving element. The rouleaux formation and aggregation of the red blood cells begin as soon as agitation ends, causing the intensity of the light passing through the blood to change over time. The waveform expressing this change in light transmission over time is called a syllectogram. At the same time, the CBC measuring unit measures HCT and MCV. The automated hematology analyzer uses a calculation method exclusive to Nihon Kohden to calculate ESR (1-hour value) based on the HCT and MCV values obtained by the CBC measuring unit and the syllabogram produced by the ESR measuring unit.[8]

To provide the method comparability between the automated method and the standard method, the International Council for Standardization in Hematology and Clinical (ICSH) and Laboratory Standards Institute (CLSI) recommended that new methodologies are needed to standardize against its own reference method known as method comparison.[9-12]

Therefore, the study's objective was to compare the Celltac Alpha+ (MEK-1305) Automated Hematology and ESR analyzer assessment of the erythrocyte sedimentation rate to the traditional Westergren technique.

Materials and methods

Blood sample collection

A total of 220 EDTA whole blood samples were all leftovers from daily routine samples of both inpatients and outpatients from Maharaj Nakorn Chiang Mai Hospital and PROMT Healthcare Center AMS CMU, Chiang Mai, Thailand. The use of de-identified blood samples was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (exempted number AMSEC-65EM-011). Hemolyzed and clotted samples were excluded. Whole blood samples were collected in 3.0 mL tripotassium EDTA, processed both by manual Westergren method and by Celltac Alpha+ (MEK 1305) analyzer following the manufacturer's instructions, and examined within 4 hours of venipuncture.

Population study

Population study was classified as descriptive statistics, which is used to describe population features such as frequency, distribution patterns, central tendency, data range, standard deviation (SD), relative standard deviation (RSD), and standard error of the mean (SEM). Following the ICSH recommendations, method comparisons were further assessed in three subgroups, according to the ESR values obtained with the Westergren method, that is, normal (<20 mm.), high (20-60 mm.), and very high (>60 mm.) of the analytical range.

Precision study

B228N, B228L (commercial quality control materials), and a normal blood sample (ESR less than 20 mm.) were used to evaluate the intra-run precision. Each sample was examined 20 times with the MEK-1305 within a day. For the inter-run precision, B228N and B228L were measured once a day for 20 consecutive days. All the results were calculated to find the arithmetic mean, standard deviation, and % coefficient of variation (%CV) Then, %CV was compared to the total allowable error (%TEa).

Sample carryover assessment

Conforming to the CLSI H26-A2 guidelines, any analyzers that utilized a single capillary were required to assess the carryover between samples with high results and low results. The MEK-1305 was evaluated for carryover because it aspirates a specific volume of blood into a capillary that is used to analyze all samples, followed by a washing cycle. Samples were assessed in triplicate for both the high target value (HTV) and the low target value (LTV). % Carryover was calculated using the following formula.

Comparative study

This study performed statistical calculations using MedCalc and IBM SPSS. Three statistics were involved: Spearman's rank correlation coefficient, Passing-Bablok linear regression, and the Bland-Altman mean difference

plot for the method comparison study. Bias, accuracy, and limits of agreement were derived using the Bland-Altman plot.

Results

Population study

According to the population study, the average age of the 220 patients was 52.95 years old. The author determined that the three age groups were compatible with the ESR reference range. The author concluded that three age groups i.e., 0-50 years old, 51-85 years old, and older than 85 years old, agreed with the reference range of ESR. It showed that most patients were between the ages of 51 and 85. In addition, it also showed that most of the patients were women (60.5%) (Table 1)

Table 1. Basic statistical information of population characteristics.

Characteristics	Study population (N=220)
Mean age (years)	52.95
Age groups	
0-50 (years)	92 (41.8%)
51-85 (years)	125 (56.8%)
>85 (years)	3 (1.4%)
Gender	
Male	87 (39.5%)
Female	133 (60.5%)

Initial analysis

A total of 220 samples were processed on Celltac Alpha+ (MEK-1305) automated hematology and ESR analyzer compared to the standard Westergren method. The standard method yielded minimum, maximum, and mean ESR values of 0 mm/hr, 140 mm/hr, and 22.6 mm/hr, respectively. In contrast, the automated method yielded minimum, maximum, and mean ESR values of 1 mm/hr, 119.7 mm/hr, and 20.7 mm/hr, respectively.

Individually, the analysis revealed that ESR values in approximately 70.5% of patient specimens (N=155) were within the reference range (1 to 20 mm/hr) defined as "Normal ESR" and approximately 26.5% of patient specimens (N=65) were beyond the reference range. Results were based on the standard Westergren method. Analyzed results are shown in Table 2.

Table 2. ESR values of a sample of 220 cases categorized according to ESR (mm/hr) ranges when analyzed using the automated analyzer (MEK-1305) compared to the standard Westergren method.

ESR (mm/hr)	N (%)	Method	Min to Max	Mean (95%CI)	SD	RSD	SEM
1-20	155 (70.5)	Westergren	1-20	10.5 (9.6-11.3)	5.627	0.528	0.452
		MEK-1305	1-27	9.9 (8.9-10.9)	6.291	0.634	0.505
21-60	50 (22.7)	Westergren	21-60	36.4 (33.3-39.5)	10.921	0.299	1.545
		MEK-1305	16.7-54.3	32.8 (29.5-36.0)	11.418	0.348	1.615
>60	15 (3.8)	Westergren	70-140	95.6 (82.5-116.5)	23.709	0.248	6.1216
		MEK-1305	66-119.7	92.0 (81.1-102.9)	19.709	0.214	5.089
Total	220	Westergren	1-140	22.2 (18.9-25.4)	24.449	1.103	1.648
		MEK-1305	1-119.7	20.7 (17.6-23.8)	23.361	1.128	1.575

Precision study

Normal blood samples and commercial quality control materials (B228N and B228L), developed by Nihon Kohden, had been used to carry out a precision study. In an intra-run precision, %CV of 8.12 and 2.01 were obtained, while %CV of 7.6 and 2.9 were obtained in an inter-run precision. Moreover, intra-run precision results obtained by analyzing normal blood samples (sample 1) showed a good level of precision below 10% (CV 7.7%). It could be concluded that the variations that occurred were acceptable according to the %CV value assigned by the manufacturing company (%Total allowable error < 10%). Analyzed results are shown in Table 3.

Table 3 Intra-run and Inter-run precision of Celltac Alpha+ (MEK 1305) analyzer.

	Intra-run precision (N=20)		Inter-run precision (N=20)		
	B228N (Normal)	B228L (Abnormal)	Sample 1	B228N (Normal)	B228L (Abnormal)
Mean±SD	7.5±0.6	54.6±1.1	21.2±1.6	6.6±0.5	52.8±1.5
% CV	8.12	2.01	7.7	7.6	2.9

Sample carryover assessment

High target value samples (HTV) and low target value samples (LTV) were measured three times each by Celltac Alpha+ (MEK-1305). The study showed that no contamination happened between the process with %Carryover = 0.0%

Statistical analysis for method comparison

A total of 220 EDTA whole blood samples were processed and analyzed to compare each method statistically. As mentioned in the previous topic, data were categorized into specific parameters and intervals.

Spearman's rank correlation coefficient

The analysis indicated a positive correlation between the standard Westergren method and the automated method. All ranges (N=220) had ρ=0.916 (95%CI: 0.892 to 0.935). Additionally, the highest ρ was given in the low % Hct parameter (ρ=0.969, 95%CI: 0.938 to 0.984). The analyzed results are shown in Table 4.

Table 4 Method comparison results between ESR measurement by Westergren and Celltac Alpha+ (MEK 1305).

		Spearman's rank Correlation		Passing-Bablok linear regression					Bland-Altman	
		ρ	Equation	Slope	Intercept	Mean bias	SD	1.96SD	Upper LOA	Lower LOA
	All ranges (N=220)	0.916	Y = 0.963x - 0.815	0.963	-0.815	-1.446	-4.631	-9.076	-10.523	7.630
ESR (mm/hr)	1-20 (N=155)	0.783	Y = 1.100x - 1.300	1.100	-1.300	-0.529	-3.971	-7.784	-8.313	7.255
	21-60 (N=50)	0.891	Y = 1.023x - 4.912	1.023	-4.912	-3.65	-4.548	-8.913	-12.565	5.261
	>60 (N=15)	0.922	Y = 0.902x + 5.920	0.902	5.912	-3.5733	-7.619	-14.932	-18.506	11.359
Hct (%)	<35 (N=35)	0.969	Y = 0.977x - 1.262	0.977	-1.262	-2.171	-6.281	-12.311	-14.482	10.139
	35-45 (N=135)	0.874	Y = 0.926x + 0.111	0.926	0.111	-1.155	-4.600	-9.018	-10.172	7.863
	>45 (N=52)	0.655	Y = 0.833x - 0.350	0.833	-0.350	-1.704	-3.217	-6.305	-8.009	4.601
MCV (fL)	<80 (N=45)	0.892	Y = 0.997x - 1.661	0.997	-1.661	-2.424	-5.503	-10.785	-13.210	8.361
	≥80 (N=175)	0.919	Y = 0.958x - 0.617	0.958	-0.617	-1.195	-4.361	-8.548	-9.743	7.354

Passing-Bablok linear regression

To evaluate the relationship between the two methods, it indicated a systematic difference, which is described by a linear regression equation y = 0.963x - 0.815 at 95%CI, as represented in Table 4 and Figure 1A. A slope was used to determine if the slope equals 1; the hypothesis would only be accepted if the 95% confidence interval contained 1. The experiment revealed that the slope range was 0.933 and 1.000, which consisted of 1, indicating no statistically significant difference between the slope and 1. Next, the y-intercept was used to determine if the intercept equals 0; the hypothesis would only be accepted if the 95% confidence interval contained 0. The experiment revealed that the range of the y-intercept was -1.300 and -0.150, which does not consist of 0, indicating a statistically significant difference, which meant the y-intercept of the linear regression equation was unacceptable. The possible cause of the unacceptable y-intercept might come from the specific principle that Celltac Alpha+ The MEK-1305 used, leading to a lower result than the standard Westergren method; this is correlated to the prior research of the others.

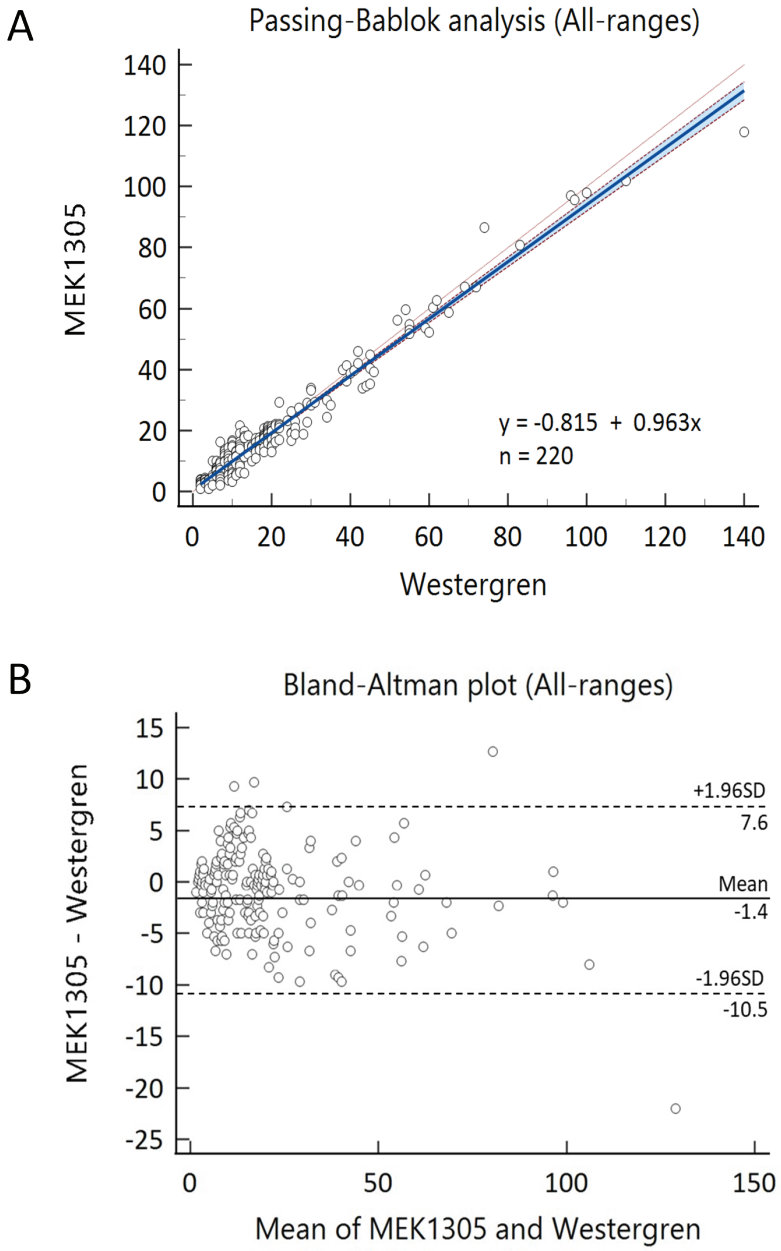

Figure 1. *Comparison between erythrocyte sedimentation rate values using Celltac Alpha+ (MEK 1305) analyzer against the Westergren method (n=220). A: Passing-Bablok scatter diagram with an equation y=-0.815 (95% CI: -1.300 to -0.150) + 0.963 (95% CI: 0.933-1.000)x, B: Bland–Altman scatter diagram with a mean bias of -1.4 (95% CI: -0.8 to 2.1), the value of 7.6 for the upper limit and -10.5 for the lower limit can be considered acceptable limits, implicating a non-significant bias based on a clinical criterion.*

Bland-Altman mean difference plot

The result represented mean bias =-1.4 (95% CI: -0.8 to 2.1), upper limit of agreement (Upper LOA) =7.6 (95% CI: 6.6 to 8.7), and lower limit of agreement (Lower LOA) =-10.5 (95% CI: -11.6 to -9.5) when measuring agreement between two qualitative measurements in 220 samples (Table 4 and Figure 1B). Additionally, negative mean biases could be observed in those parameters that are not considered normal. However, more blood samples should be collected to carry out the analysis effectively since there were not enough samples in some ranges of parameters.

Discussion

The erythrocyte sedimentation rate (ESR) is a commonly used laboratory test in clinical settings. It measures the rate at which red blood cells settle in a specific pipette within a defined interval, typically observed over 1 hour. ESR is an informative indicator for inflammatory conditions such as rheumatoid arthritis, giant cell arthritis, polymyalgia rheumatica, and other connective tissue disorders. Advancements in the study of the sedimentation phenomenon have led to the automation of ESR testing, resulting in the development of numerous automated instruments now commonly

utilized in routine clinical practice. In this study, in line with ICSH recommendations, we assessed the analytical performance of the Celltac Alpha+ (MEK-1305). This new automated ESR analyzer operates on the sedimentation principle using undiluted EDTA samples.

A nonnormal distribution resulted from a population study comparing ESR results obtained by the automated analyzer and the standard Westergren method. Of 220 EDTA whole blood samples, 60.5% were female, and 52.95% were aged between 51 and 85. The utilization of the MEK-1305 yielded a lower result (20.7 mm./hr.), whereas the standard Westergren method provided a higher result (22.2 mm./hr.). These differences occurred due to a contrasting principle between these methods. The automated analyzer utilizes the principle of measuring Rouleaux formation of red blood cells and then calculates it into the red blood cell sedimentation rate. Consequently, this may result in different values.

This study aligns with the previous research by Manoj A. Kahar and colleagues,[13] which found that automated analyzers yield lower test results than the standard Westergren method. From the initial statistical analysis of the entire sample of 220 cases, the minimum ESR value obtained from the standard Westergren method was 2 mm./hr., whereas from the automated analyzer, it was recorded as 1.0 mm./hr. This discrepancy may have arisen due to clerical errors during the operators' interpretation of the result. Additionally, it was observed that in blood samples with hematocrit less than 35% and an MCV less than 80 fL, there was a tendency toward higher than normal ESR values. Conversely, blood samples within the normal range of hematocrit and MCV also showed a tendency for ESR values within the normal range.

The precision study used normal blood samples from volunteers and quality control materials at 2 levels, namely B228N (Normal ESR level) and B228L (Abnormal ESR level). The intra-run precision study found that in B228N, the %CV was higher than the %CV of B228L (%CV, B228N=8.12%. %CV, B228L=2.01%). This aligns with previous studies by C. Cha *et al*.,[14] Nihal *et al*.,[15] and Ivana Lapic et al.[16] Due to the superior physicochemical characteristics of the quality control sample compared to blood samples from individuals, this results in a higher %CV for Sample1 when compared to B228N and B228L. This reference is based on the study conducted by Plebani and Piva.[17] The study on inter-run precision also revealed that the %CV of B228N was higher compared to the %CV of B228L. This finding aligns with previous research by A. Kahar *et al*.,[13] Mahlangu and Davids[18], Horsti *et al*.,[19] and Schapkaitz *et al*.[20] However, it's important to note that based on the study results, the %CV obtained exceeded the %TEa criterion set by the company at %TEa=10%. Moreover, the study revealed that this automated analyzer does not exhibit sample carryover, with the calculated %Carryover =0.0%. This finding aligns with the earlier study of Ryosuke Maki and colleagues in 2021.[21] The statistical analysis using Spearman's rank correlation coefficient within the entire sample of 220 cases revealed a positive correlation. Additionally, it was observed that the trend of the ρ value increased in samples with higher ESR values, lower hematocrit values, and higher MCV values. From the Passing-Bablok linear regression analysis, it was observed that the slope did not meet the criteria. This might be because the Celltac Alpha+ (MEK-1305) Automated Hematology and ESR analyzer tends to provide lower ESR values than the Westergren method. Consequently, this affects the y-intercept of the equation, which does not meet the criteria. This finding is consistent with previous studies. Moreover, when the groups were divided based on ESR values (mm/hr) into different ranges: normal range (1 to 20 mm/hr), high (21 to 60 mm/hr), and very high (>60 mm/hr), it was found that the criteria were met only in the very high ESR group. This might be attributed to a smaller number of samples in the very high ESR group, leading to this outcome. From the Bland-Altman mean difference plot analysis across the entire sample of 220 cases, it was observed that there is consistency in all parameters. However, a relatively high mean bias was noted for the ESR parameter within the range of ESR values between 21 and 60 mm/hr and for ESR values greater than 60 mm./hr. The mean bias was recorded as -3.7 (95% CI: -4.9 to -2.4) and -3.6 (95% CI: -7.8 to 0.6), respectively.

Based on the statistical analysis of the three testing statistics, this automated analyzer can be effectively utilized in clinical settings, provided that the manufacturer's guidelines are adhered to accurately.

Conclusion

Celltac Alpha+ (MEK 1305) analyzer showed good correlation with the conventional Westergren method and an acceptable bias over the entire range of ESR, exhibiting satisfactory concordance of ESR results between Celltac Alpha+ (MEK 1305) and reference Westergren method. Celltac Alpha+ (MEK 1305) analyzer offers major advantages such as use of EDTA sample, reduced sample volume, ease of performance, reduction in biohazard risk, and reliability, making it a valid substitute for reference Westergren method for ESR determination.

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