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### Research Article

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# Is Vision C interchangeable with the modified Westergren method for the erythrocyte sedimentation rate?

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### Abstract

**Objectives:** As one of the most widely used tests, the erythrocyte sedimentation rate (ESR) is a measurement of sedimentation reaction in blood samples. Although the recommended method for ESR measurement is the Westergren method, this method has some disadvantages in comparison with automated ESR systems. In this cross-sectional study, we aimed to compare Vision C with the Westergren method.

**Methods:** The Vision C automated ESR system and the modified Westergren method were compared using  $K_3$ EDTA-containing tubes and citrated blood tubes from randomly selected 100 patients. Precision, bias, and interference calculations were performed.

**Results:** The mean difference between the Vision C (room temperature) and the modified Westergren method was  $22.8 \pm 26.6$  mm/h (95% CI for the mean was 17.50-28.08 mm/h). The mean difference between the Vision C (18 °C) and the modified Westergren method was  $27.4 \pm 30.6$  mm/h (95% CI for the mean was 21.3-33.5 mm/h). The calculated regression analysis equation was "y=0.263+1.053x" and "y=-0.530+0.851x" for the Vision C (room temperature) and the Vision C (18 °C), respectively. The imprecision values

ranged at 7.55–17.09%. According to our external quality control results, bias was 11.11 and 9.66 for the low- and high-level samples, respectively.

**Conclusions:** The Vision C automated ESR system has a comparable analytical performance with the modified Westergren method. The Vision C automated system may be utilized in ESR measurements with quality control evaluations. Nevertheless, temperature correction using Manley's monogram causes an important negative bias and should be taken into consideration during the evaluation of the Vision C results.

**Keywords:** erythrocyte sedimentation rate; method acceptability; Vision C; Westergren.

# Introduction

In clinical medicine, the erythrocyte sedimentation rate (ESR) is one of the most conventional and most widely used tests throughout the world, and it is defined as "the length of sedimentation reaction in blood" [1]. To analyze the ESR, a distance measurement between the surface meniscus and the upper limit of the erythrocyte column is performed after a standard waiting period [2].

The ESR is determined after 1 h in a vertically placed blood tube. The major effect on erythrocyte sedimentation is the formation of erythrocyte aggregates, which are also called rouleaux or clumps. Aggregation is the first phase in the erythrocyte sedimentation process, followed by sedimentation and packing to complete the process [2, 3].

The ESR result, a beneficial tool for assessing acute phase inflammation, is employed as a useful marker of infectious disease [4, 5]. As a predictor of inflammation, the ESR results can be used in estimating several diseases such as stroke [6], diabetes mellitus [7], and coronary heart disease [8]. Moreover, it is helpful in the diagnosis and follow-up of several diseases, including rheumatoid arthritis [9], giant cell arteritis [9], polymyalgia rheumatic [10], and malignancies [11, 12].

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To date, several ESR measurement methods have been developed. Although the recommended method for ESR measurement and the preferred method for new method validation is the Westergren method, this method has some disadvantages such as infection risk, difficult analysis process, and long analysis time in contrast with the automated ESR [3, 13]. The new automated ESR systems have a comparable analytical performance with the Westergren method [3, 14, 15]. Moreover, these systems have several advantages, such as automated measurements, keeping results in check with the internal and external quality control samples, same sample for both ESR and complete blood count, and saving on time and staff [3, 16].

Vision C is an automated measurement system in which the ESR is analyzed with a K<sub>3</sub>EDTA blood sample. In this study, we aimed to compare Vision C and the modified Westergren method and to evaluate the analytical performance of Vision C. To the best of our knowledge, this study is the first to investigate the analytical performance of the Vision C ESR system.

# Materials and methods

### Collection of blood samples

In this study, Vision C (YHLO Biotech, Shenzen, China) and the modified Westergren method were compared using K3EDTA-containing tubes (Greiner Bio-One International, Frickenhausen, Germany) and citrated blood tubes (Becton Dickinson System, New Jersey, USA). Recommendations of International Council for Standardization in Hematology (ICSH) were used to compare two ESR system [17]. ICSH suggests at least 30 samples in the analytical range for comparison. In this study, a total of 100 outpatients were randomly selected from the Ministry of Health Ankara City Hospital. Blood collection was performed with two types of blood tubes simultaneously on all 100 patients. All samples were analyzed within 4 h of venipuncture. The ESRs were measured using both the Vision C automated system and the modified Westergren method. Within-run impressions were calculated using the samples with low, medium, and high erythrocyte sedimentation values. All procedures were approved by the local Ethics Committee, and signed informed consent forms were obtained from all the volunteers.

### Measurements of the ESRs

To achieve the automated ESR values, all the K<sub>3</sub>EDTA-containing blood tubes were loaded to the Vision C automated ESR system. The loaded tubes were mixed by turning them upside-down for about 18 times within 3 min. All phases of the ESR were measured by an optical system using infrared screening. A total 120 measurements were completed within 20 min for each sample. The last measurements were recorded as room temperature ESR values. To comply with the temperature of 18 °C, a correction was performed in all the ESR results according to Manley's monogram [18]. These results were recorded as 18 °C ESR values. All of the measurements stated as room temperature were carried out in the range of 20-22 °C in the laboratory environment where the central air conditioning is located.

To achieve the manual ESR measurements, all of the citrated blood tubes were placed in the modified Westergren set. After 30 min, the sedimentation rate was assessed visually according to modified Westergren 30 min set.

### Imprecision studies

According to ICSH protocol [17], an intra-run imprecision study was performed on the Vision C automated system with three different patient samples by performing 10 measurements during an 8 h period. The inter-run imprecision study was performed in the Vision C automated system with commercial internal quality control samples via analyzing three times a day for five consecutive days.

### Sample stability studies

For the stability studies, the samples of 32 patients were selected randomly. The ESRs were measured by the Vision C automated system at 0, 4, 6, 8, and 24 h after collection. A total of 16 samples were stored at room temperature and 16 samples at +4 °C during analyses.

### **Interference studies**

To investigate the effect of lipemia and hemolysis, total parenteral nutrition (TPN) and hemolyzing solution were used. The K3EDTA samples of 10 patients were randomly selected. The dilution rates were selected as 1/100 and 1/10 for the lipemia and hemolysis interference studies, respectively. Simultaneously, serum physiologic was used at the same dilution concentration with the TPN and hemolyzing solution. The samples without and with pre-treatment were analyzed. The results were recorded as "neat" for those without pretreatment and "spiked" with those with pretreatment.

### **Bias studies**

For the bias estimation, an external quality assurance (One World Accuracy, Fraserwood Court Burnaby, Canada) was performed for two different levels of quality control samples.

### Reference interval verification studies

For verification of reference interval, 20 healthy women and 20 healthy men's samples were analyzed and checked if it is within our reference range.

### Statistical analysis

The descriptive statistics and the Kolmogorov-Smirnov test for the normality of the distribution were reported for each variable. The Wilcoxon signed ranks test was used to compare the variables, and Spearman's rank correlation test was used to evaluate the correlation among the different methods because of the non-parametric distribution of all the variables. A linear regression analysis was performed according to the Passing-Bablok approach. The bias and limits of the agreement were performed using the Bland-Altman analysis. The calculations were performed using the MedCalc statistical software (ver. 12.3.0.0.; Belgium) and SPSS 13.0 for Windows software (Chicago, IL). Values of p<0.05 were accepted as statistically significant.

# Results

# Results of the imprecision studies

When measuring at room temperature, for low-, middle-, and high-level ESR samples, intra-run imprecision values were 17.2, 13.9, 2.89%, respectively. When measuring at 18 °C, for low-, middle-, and high-level ESR samples, intra-run imprecision values were 18.1, 14.9, 3.35%, respectively.

For the low- and high-level control samples, the inter-run imprecision values were 11.3 and 5.91%, respectively, for room temperature and 14.6 and 4.76%, respectively, for 18 °C.

# Results of the stability studies

After storing 4, 6, 8, and 24 h at room temperature, blood tubes were reanalyzed. For room temperature, the ESR results of the 24th h were lower than the baseline values. For +4 °C, the ESR results of the 6th, 8th, and 24th h were higher than baseline values. The results of the stability experiments are shown in Table 1.

### Results of the interference studies

After analyzing without and with pre-treatment samples, there was no difference in results of lipemic and hemolytic samples compared to without pre-treatment samples (p>0.05). The results of the interference experiments are shown in Table 2.

Table 1: Bold values show significant difference.

## Results of the bias studies

According to the data of the external quality assurance program, bias was estimated at 11.11 and 9.66% for the low- and high-level external quality control samples, respectively.

# Results of reference interval verification studies

According to results of 40 healthy persons, all ESR values were within our reference range.

# Results of the method comparison study

The accuracy of the ESR measurement of Vision C was established with respect to ICSH protocol. The ESR was measured in all the K3EDTA blood samples by Vision C and in the citrated blood tubes by the modified Westergren method. The mean  $\pm$  SD ESR value was 28.5  $\pm$  30.8 mm/h (95% CI for the mean was 22.4-34.7 mm/h) for Vision C (room temperature), 22.8  $\pm$  26.6 mm/h (95% CI for the mean was 17.5-28.1 mm/h) for Vision C (18 °C), and 27.4  $\pm$  30.6 mm/h (95% CI for the mean was 21.3-33.5 mm/h) for the modified Westergren method.

The correlation coefficients were 0.973 (p<0.001) and 0.976 (p<0.001) between the modified Westergren and Vision C (room temperature) and between the modified Westergren and Vision C (18 °C), respectively.

The Passing-Bablok regression analysis yielded the equation "y = 0.263 + 1.053x" between the modified Westergren and Vision C (room temperature) (Figure 1A) and "y = -0.530 + 0.851x" between the modified Westergren and Vision C (18 °C) (Figure 1B). The Vision C (room temperature) ESR method (n=100) yielded a slope of 1.053 (95% CI, 1.00-

	Baseline (n=16)	4 (n=16)	6 (n=16)	8 (n=16)	24 (n=16)
ESR values for room tmperature					
Mean $\pm$ SD, mm/h	$23.88 \pm 24.31$	$23.13 \pm 25.37$	$24.06 \pm 25.39$	$25.50 \pm 25.14$	$12.75 \pm 19.84$
Median, min-max	13 (3-77)	9.5 (3-84)	8.5 (3-77)	7 (3-77)	5.5 (3-75)
Mean of differences, mm/h		0.75	-0.19	0.38	11.13
95%CI, mm/h		-0.73 to 2.23	-3.93 to 3.56	-3.56 to 4.31	3.64-18.61
p-Value		0.296	0.916	0.842	0.003
ESR values for 4 °C					
Mean $\pm$ SD, mm/h	$9.81 \pm 7.90$	$9.81 \pm 7.42$	$15.25 \pm 13.44$	$14.50 \pm 12.71$	15.56 ± 12.83
Median, min-max	6.5 (3-30)	6.5 (3-29)	12 (3-47)	12 (3-46)	12.5 (3-44)
Mean of differences, mm/h		<0.001	-5.43	-4.69	-5.75
95% CI, mm/h		-0.67 to 0.67	−9.85 to −1.03	-8.50 to -0.88	−9.64 to −1.86
p-Value		1.000	0.019	0.019	0.007

**Table 2:** The effect of lipemia and hemolysis interferences on ESR measurements.

Parameter	Median	Minimum-maximum	p-Value
Neat lipemia	12	5-37	0.594
Spiked lipemia	11.5	5-24	
Neat SF	8.5	4-27	0.065
Spiked SF	12	5-27	
Neat hemolysate	12	5-46	0.395
Spiked hemolysate	12.5	5-28	
Neat SF	12	5-46	0.944
Spiked SF	11	5-52	

1.084) with an intercept of 0.263 (95% CI, -0.253 to 1.000) (p=0.21). The Vision C (18 °C) ESR method (n = 100) yielded a slope of 0.851 (95% CI, 0.810-0.889) with an intercept of -0.530 (95% CI, -1.111 to -0.240) (p=0.06).

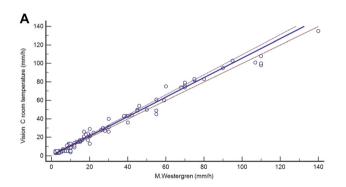
The agreement between the results obtained by the different methods was demonstrated in different plots according to Bland–Altman. The mean values were –1.1 mm/h (limits of agreement, –9.5 to 7.2 mm/h) for the modified Westergren and Vision C (room temperature) (Figure 2A) and 4.6 mm/h

(limits of agreement, -7.3 to 16.5 mm/h) for the modified Westergren and Vision C (18  $^{\circ}$ C) (Figure 2B).

# **Discussion**

As a simple definition, ESR is a numerical value in mm per hour obtained by measuring the distance between the lowest point of the surface meniscus to the upper limit of the sediment in a column of anticoagulated and diluted blood [13]. Although the recommended method for the ESR measurement is the Westergren method, several requirements, such as an automated and closed system, rational use of human resources, acceptable analytical imprecision and combination of ESR, and reticulocyte and complete blood count blood tubes, have emerged over time [16].

Recently, several automated systems analyzing ESR using the same blood tube with complete blood count have been produced. Whether these results are compatible with the reference method is still being investigated. In one study, the analytical performance of the Ves-Matic Cube



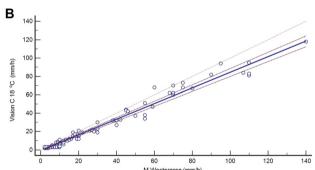
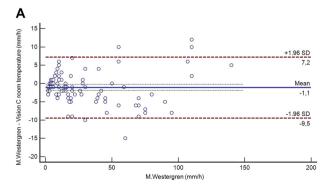
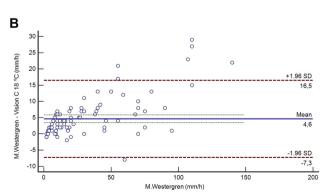


Figure 1: (A) Comparison of two methods for ESR measurement: Vision C (room temperature) and modified Westergren method. (B) Bland-Altman plot of the difference between ESR values obtained with modified Westergren method and Vision C (room temperature) against the mean of ESR values in patients blood samples.





**Figure 2:** (A) Comparison of two methods for ESR measurement: Vision C (18 °C) and modified Westergren method. (B) Bland-Altman plot of the difference between ESR values obtained with modified Westergren method and Vision C (18 °C) against the mean of ESR values in patients blood samples.

200, an automated ESR system, was evaluated, and it was demonstrated to have a comparable analytical performance with the Westergren method even if temperature correction was applied [15].

In another study comparing the Ves-Matic Cube 200 with the Westergren-based diluted methods using 4 vols blood plus 1 vol citrate, the Ves-Matic Cube 200 showed a poor correlation with the ICHS reference method, and the Westergren method had an important negative bias at low ESR levels and a random bias at high ESR levels [3].

Micro Test 1, an automated ESR system, was found to have a comparable analytical performance with the Westergren method [19].

Plebani and Piva [16] investigated the analytical performance of TEST1<sub>EC</sub>, an automated ESR system, using an aspirated blood sample from an EDTA-anticoagulated tube. The authors found a significant difference between the TEST<sub>1EC</sub> and the Westergren method. In another study, the Alifax Test 1 system was found to not be a reliable alternative for the Westergren method for high ESR levels [20].

ESR was investigated in terms of inflammatory performance, and TEST 1 (Alifax) was found to have a better performance than the Westergren method using blood inflammatory protein concentrations [21] Moreover, Alifax could be used to determine the ESR level of rheumatoid arthritis patients [22].

Vision C is an automated ESR system in which the measured room temperature ESR results are converted to 18 °C by calculating Manley's monogram [18]. In the current study, we reported that the Vision C (room temperature) ESR results were comparable with the modified Westergren method. It has some advantages, such as laboratory safety, quality applications, full automation, and reduced need for trained personnel and excessive blood sample.

In our study, the most striking finding was the presence of an important negative bias in the Vision C (18 °C) ESR results in comparison with the modified Westergren method. Therefore, we recommend reporting the ESR results without correcting them to 18 °C to present a comparable result with the modified Westergren method. Additionally, laboratories using temperature correction according to Manley's monogram should be verified their ESR results.

Undiluted samples collected in K<sub>3</sub>EDTA-containing tubes have a major advantage for ESR measurements. Red blood cell aggregation and rouleaux formation have an important effect on ESR measurement. The ESR samples with K<sub>3</sub>EDTA have a preserving effect on the stability and morphologic features of cells. Additionally, a

preanalytical mistake may occur in the altered bloodsodium citrate ratio because of the partially coagulated specimen. Test refusal or preanalytical errors can be prevented by the specimens with K<sub>2</sub>EDTA [16]. We collected all the samples with K<sub>3</sub>EDTA-containing tubes from the patients.

The hematocrit level is a significant confounding factor in ESR measurements [3]. Curvers et al. [3] established that samples with more than 20 mm/h ESR values had considerably lower ESR values in the Westergren reference method. In our study, Vision C did not correct for hematocrit.

In recent years, medical laboratory directors have made a great deal of effort to attain quality assurance and accreditation. In an automated ESR system, internal and external quality control samples are presented to medical laboratories. We calculated the within-day and betweenday precision values using internal quality control results. The results ranged at 7.55–17.1%. According to our external quality control results, bias was 11.1 and 9.66 for the different level samples. As there was no allowable total error value for ESR, we did not interpret the mentioned values about our system.

Our study has some limitations. First, we used the modified Westergren method as the reference method. Second, our blood samples had predominantly low and medium ESR levels. We obtained only a few high ESR level blood samples. Third, we did not investigate the effect of hematocrit on the ESR measurement. Lastly, we did not examine the clinical correlation of the results, and this condition could cause an underestimation if the temperature corrected results have an advantage in the Westergren method for different patient types.

In summary, the ESR results obtained with the Vision C automated ESR system are comparable with those of the modified Westergren method. Nevertheless, temperature correction using Manley's monogram causes an important negative bias unlike in the Westergren method. Comprehensive studies to further investigate the clinical correlation of the ESR results with other inflammatory markers are required.

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**Competing interest:** Authors state no conflict of interest. **Informed consent:** Informed consent was obtained from all individuals included in this study.

Ethical approval: All procedures were approved by the local Ethics Committee.

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