



# **Correlating Automated IRIA ESR Analyzer and Westergreen Method for Determination of Erythrocyte Sedimentation Rate**

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The erythrocyte sedimentation rate (ESR), or sedimentation rate (sed rate), is a measure of the settling of red blood cells in a tube of blood during one hour. The rate is an indication of inflammation and increases in many diseases. Westergreen method is routinely used determination of ESR, however, it requires large volume of blood and it is time consuming as it takes one hour for analysis. In order to overcome these challenges, new technologies have implemented an automated system which saves on labour, no need for aliquots, shorter turnaround time and minimizes exposures of laboratory staff to biohazard risks. The main objective of this study was to compare Westergreen method and automated IRIA analyzer in determination of ESR. The specific objectives were to determine the ESR using both Westergreen tube method and automated IRIA analyzer and to determine the correlation between Westergreen tube method and automated IRIA analyzer methods. This cross-sectional study was carried out in 205 blood samples at Polyclinique La Medicale from August 2017 to April 2018. Data were analyzed using SPSS, version 21. The current

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study included a total of 205 participants 123 (60%) females and 82 (40%) males. For a total of 205 participants, the normal tests on westergren method were 142 (69.3%) and abnormal tests were 63 (30.7%), whereas for automated IRIA ESR analyzer, 131 (63.9%) were normal and 74 (36.1%) tested abnormal. There was a strong correlation between automated IRIA ESR analyzer and Westergreen method with  $r=0.9$ . The authors recommend that IRIA ESR analyzer should be used in determining ESR due to its advantages over Westergreen method.

*Keywords: Correlation; Westergreen method; automated IRIA ESR analyzer; ESR.*

## 1. INTRODUCTION

Erythrocyte sedimentation rate (ESR) is an hematological test used to investigate different diseases. It is still the most reliable and gold standard test in most clinical facilities [1]. New documents were published by the International Council for Standardization in Hematology and the National Committee for Clinical Laboratory Standardization (NCCLS) (ICSH). In 1988, both NCCLS and ICSH published new guidelines for quality assurance and in 1993, ICSH group published also new recommendations, highlighting the importance of ensuring that measurements obtained in different laboratories were comparable [2]. Several new methods of which some of them are automated or semi-automated, became available in 2001 [3]. For the purpose of improvement, the ICSH and Clinical Laboratory Standards Institute (CLSI) made and published new recommendations in 2010 and 2011. For ESR determination, the standard document stated that all new technologies, instruments, or methodologies had to be evaluated and compared against the Westergreen reference method before being introduced into clinical use. In addition to that the manufacturers provide a protocol on the reliability and trueness of any method and instrument, as well as calibration and control procedures for any new method [4,5,6].

It is evident that till now, there are still significant variations in the methodology used to determine ESR, However NCCLS, CLSI and ICSH published methods for standardizing performance of the ESR and the Westergreen method was confirmed to be a reference method as it is reliable, reproducible, and sensitive with results reported in millimeter sedimentation after 60 minutes [7].

Westergreen method determines erythrocyte sedimentation after 1 hour in a vertically mounted tube of defined length and bore size, thereby analyzing all 3 phases in the process of

erythrocyte sedimentation: aggregation, sedimentation, and packing [8]. It carries a risk of infection (open tubes), needs relatively large volumes of blood, and, with an analysis time of 1 hour, hence it is time-consuming. According to international council of standardization in hematology, westergreen tube method has a prolonged turn around time, it is conceived in a system of open tube which exposes to biohazard risk [4,9,10].

The advantages of integration of ESR technology into automated systems include savings on labor work, no need for aliquots and therefore more efficient use of sample volumes, shorter turnaround times, and minimal exposure of laboratory staff to biohazards, however its disadvantages include possible higher costs of instrumentation [5]. At least 30 samples spanning the analytical range of the instrument should be compared [9]. Although, westergreen tube technique has many disadvantages, in Rwanda, there is no other advanced method that was developed or validated for overcoming these disadvantages. Hence, this study was of great importance to be conducted. The general objective of this study was to correlate automated IRIA analyzer and Westergreen method for determination of erythrocyte sedimentation rate.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

The current study was conducted at POLYCLINIQUE LA MEDICALE located near Centre Saint PAUL, at Nyarugenge district in Kigali City, Rwanda. A cross-sectional study was carried out on patients who attended Polyclinique LAMEDICALE during the period from August 2017 to April 2018. Citrated (Seditainer ESR tubes, Becton Dickinson) or EDTA-anticoagulated (in Vacutainer tubes, Becton Dickinson) whole blood were used to determine the ESR within 4 hours after blood collection.

ESR from 205 citrated blood samples was determined using both Westergreen method and automated IRIA ESR analyzer. Samples were obtained using convenience sampling technique. During ESR determination, the ESR tubes were placed on the Westergreen rack and the red blood cells were allowed to settle for an hour, after which the ESR was measured in mm/hour. At the same time, the IRIA well of an analyzer was filled with blood sample and analyzed automatically in the automated IRIA analyzer. The results of ESR were determined in mm/hr. Results were kept using file records as well as electronically for better management of the data. The results obtained from both ESR testing methods were compared and further analysis was made. In addition, Westergreen method served as the gold standard reference method according to approved standards of hematology regarding ESR determination in clinical setting. The correlation between ESR results obtained from two different testing approaches was determined by applying Karl Pearson's formula. Procedure for computing the correlation coefficient (Young et al. 1999).

The value of correlation coefficient 'r' ranges from -1 to +1

If  $r = +1$ , then the correlation between the two variables is said to be perfect and positive

If  $r = -1$ , then the correlation between the two variables is said to be perfect and negative,

If  $r = 0$ , then there exists no correlation between the variables *value strength of correlation*

$r^2 = 0$  no correlation,  $0 < r^2 < 0.25$  very weak correlation,  $0.25 \leq r^2 < 0.50$  weak correlation

$0.50 \leq r^2 < 0.75$  moderate correlation,  $0.75 \leq r^2 < 0.90$  strong correlation

$0.90 \leq r^2 < 1$  very strong correlation

$r^2 = 1$  perfect correlation

## 2.2 Ethical Consideration

This study has been revised and approved by a departmental Institutional Review Board committee within the school of Health Sciences of Mount Kenya University, Kigali. Ethical approval has been requested from research committee Polyclinic La Medicale. To assure confidentiality, the new Numbers were used as study ID instead of names or hospital ID on patient data extraction forms.

## 3. RESULTS

### 3.1 Demographic Characteristics of Study Subject

Table 1 shows that, among all participants 123 (60%) were females and 82 (40%) were males.

The majority being between 45 and 54 years old and the minority being above 55 years old.

### 3.2 Abnormal and Normal Results from Both Automated IRIA ESR Analyzer and Westergreen Methods

Table 2 shows the results got from both IRIA analyzer and Westergreen method. Among 205 patients tested for Erythrocyte Sedimentation Rate, using IRIA analyzer, 74 (36.1%) were having elevated ESR (abnormal values indicating possible inflammatory diseases,  $ESR > 20$ mm/hour) whereas 131 people were having normal values. On the other hand Westergreen method, abnormal results were 63 (30.7%) and 142 (69.3%) of people were having normal values.

Mean average of ESR values using Westergreen method and automated IRIA analyzer.

**Table 1. Demographic characteristics of the study participants**

Age	Gender		Total
	Females	Males	
16-24	22	13	35 (7%)
25-34	31	12	51 (24.8%)
35-44	22	20	38 (18.5%)
45-54	30	20	42 (19%)
55+	18	17	39 (19%)
Total	123 (60%)	82 (40%)	205 (100%)

**Table 2. ESR results from both Westergreen method and automated IRIA ESR analyzer**

Test method	Normal %	Abnormal %	Total %
Westergren %	142 (69.3%)	63 (30.7%)	205 (100%)
IRIA analyzer %	131 (63.9%)	74 (36.1%)	205 (100%)

**Table 3. Mean average of ESR using Westergreen method and automated IRIA analyzer**

Methods	N	ESR Values			
		Minimum	Maximum	Mean	SD
ESR IRIA Analyzer (in mm/hr)	205	1	113	24.09	24.46
Westergreen Method (in mm/hr)	205	1	114	23.12	25.91

**3.3 Mean Results of ESR Using Westergreen Method and Automated IRIA ESR Analyzer**

Table 3 illustrate the mean average, minimum, maximum and standard deviation of the results of ESR measured in mm/hour. The table shows that among 205 tested samples, the minimum ESR value was 1 mm/hour for both ESR IRIA analyzer and Westergreen method. The maximum ESR measured using westergreen method was 114 mm/hour whereas it was 113 mm/hr for IRIA analyzer. The mean average of ESR for Westergreen method and IRIA analyzer were 23.12 and 24.09 mm/hour respectively. The SD of ESR results were 24.46 and 25.91 for ESR IRIA analyzer and Westergreen method respectively. There were no significant difference between means using ESR analyzer and Westergreen method (P=0.1).

**3.4 Determination of the Correlation between Westergreen Method and Automated IRIA ESR Analyzer**

Using SPSS VERSION 21, the the coefficient of variation between Westergreen method and IRIA analyzer was done using Karl Pearson's formula and it was found to be 0.9 (r= 0.8939= 0.9).

**4. DISCUSSION**

The main objective of this study was to correlate the Westergreen method and automated IRIA analyzer for determination of erythrocyte sedimentation rate. There was a strong correlation between Westergreen method and automated IRIA analyzer as the r=0.9. Actually, the Correlation is a statistical measure that indicates the extent to which two or more variables fluctuate together. A positive correlation indicates the extent to which those variables increase or decrease in parallel; a negative

correlation indicates the extent to which one variable increases as the other decrease. In this case the correlation between Westergreen and automated IRIA analyzer is positive which indicates that the values of Westrgreen may increase or decrease in parallel with that of automated IRIA analyzer which implies a strong correlation.

The conventional manual Westergren method is still considered the reference method for the measurement of ESR, despite its intrinsic practical drawbacks such as risk of infection and relatively long analysis time. The introduction of Westergreen-based semi-automated methods has substantially improved the application of ESR measurement. In line with the Westergreen reference method, these automated methods dilute whole blood with citrate, measure sedimentation of erythrocytes in dedicated tubes, and, subsequently, recalculate to conventional Westergreen units.

Automated methods generate fast and reliable ESR measurements and show good correlation with the conventional Westergreen reference method. More recently developed ESR methods circumvent the need for additional dilution and thereby optimize logistical laboratory workflow, enhance operator safety, and reduce laboratory waste. The IRIA ESR analyzer is an example of such a modern automated ESR method that uses standard citrated blood sample tubes for direct measurement of erythrocyte sedimentation.

In a study that was conducted in by Sezer et al. [11]. Ves Matic Cube 200 instrument was compared with Bland-Altman analysis method using Westergren method in which the correlation coefficient was 0.82. The correlation (r) of ESR results from the SEDIsystem and the StaRRsed system with Westergren method was 0.96 [11]. This agrees with the findings from the current study where the correlation coefficient was found to be 0.9.

In a study done by Öztürk et al. [12], 2 methods namely iSed Alcor Auto-instrument and Berkhun SDM60 Auto-instrument were compared based on their consistence for measurement of Erythrocyte Sedimentation rate. The findings from this study showed a strong correlation between the two automated methods with  $r$  equal to 0.90, however the findings were not compared with westergreen methods which would have revealed the use of these automated essays over westergreen method, a gold standard method for measuring ESR.

Hashemi et al. [1] conducted a study on Erythrocyte Sedimentation Rate Measurement Using as a Rapid Alternative to the Westergreen Method. The study compared automated Micro ESR method against Westergreen method using Pearson and Spearman's coefficients. The findings showed a strong correlation between these two methods with  $r = 0.987$  and  $r^2 = 0.974$ .

A research conducted by Alexy et al. [13], computerized tube viscometer method was compared with conventional westergreen method in determination of ESR where the correlation between the two methods was 0.92. The results were generated in 4 minutes using computerized tube viscometer method compared to Westergreen method which generates ESR results within one hour.

## 5. CONCLUSION

The aim of this study was to compare analytic performances of the IRIA analyzer and Westergren-based citrated methods. From the findings of the current research project, Erythrocyte Sedimentation rates measured with both methods were compared and they revealed a strong positive correlation with  $r$  equal to 0.9. In summary, these findings indicate that IRIA ESR analyzer is reliable and suitable system for high workload clinical laboratory. It will be vital to carry out further studies in order to determine the correlation of the two methods in cases of blood disorders including Polycythaemia, Poikilocytosis, Newborn infants, Dehydration, Dengue haemorrhagic fever, and other conditions associated with haemoconcentration.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

This study has been revised and approved by a departmental Institutional Review Board committee within the school of Health Sciences of Mount Kenya University, Kigali. Ethical approval has been requested from research committee Polyclinic La Medicale.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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