

Comparison of Automated (ISED) ESR Estimation with Conventional (WESTERGREN) Method for Validation of Automated Results

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ABSTRACT

Background: ESR inspite of being considered as a non-specific hematological test is widely used for the detection of autoimmune diseases, infections and cancers. Although conventional gold standard methods are considered accurate and authentic but are having few biohazard risks and are more time consuming so are thought to be replaced by automated methods which are less time consuming and not much expensive but need to be validated by comparing them with conventional manual (Westergren) method.

Objective: To compare the ESR values by conventional ESR method i.e. Westergren method with automated ESR estimation by ISED (ALCOR) to validate the results obtained by automated method.

Study design: A Prospective cross-sectional study

Study duration and settings: Study was conducted at Pathology Department Pak Emirates Military Hospital Rawalpindi from November 2021 to April 2022.

Material and methods: All samples were processed within 1 hour of venipuncture. Hemolyzed samples were discarded. To separate EDTA sample tubes were taken and 3ml venous blood was collected in each tube respectively.

Results: 200 samples in total were used for Westergren's (Manual Method) and ISED's ESR estimates (Automated Method). Mean age of the patients was 50.74 ± 16.84 years range from 8 to 88 years, out of total 54 % patients were male and 46 % were females, 51 % patients from OPD and 49 % were admitted patients

Conclusion: The conventional Gold standard method has proven to be more efficient by CV % but p-value calculated individually as well as in all cases was statistically insignificant $> 0.05\%$.

Keywords: Automated method, Erythrocyte Sedimentation Rate, Westergren method.

INTRODUCTION

Following inflammation certain acute phase proteins are released from the liver with concomitant rise in their plasma concentration e.g. C-reactive protein, Serum Amyloid, Albumin and to a lesser extent Haptoglobin with fibrinogen and certain complement proteins. This is known as acute phase response. It is thought to be potentiated by Interleukin - 6 release from monocytes which subsequently leads to production of prostaglandins E2 and Interleukin - 1⁽¹⁾.

Inspite of being considered as a non-specific diagnostic test, ESR is being widely used for monitoring certain diseases e.g. HIV, Tuberculosis, acute viral hepatitis, Bacterial endocarditis as well as certain malignancies e.g. multiple Myeloma and Hodgkin's disease. The most widely used method for measuring ESR is Westergren method introduced in 1921 by Westergren⁽²⁾. It is recommended by international council for standardization in haematology^(2,3) and National Committee for Clinical Lab Standards^(2,3,4)

Although, several automated methods are being widely used for estimating ESR but the available automated method ISED (ALCOR) is expected to give results which are equivalent in authenticity to Gold Standard Westergren method.

The automated method use small blood volume and can produce results in less time i.e. in just 20 sec. compared to 30-60 min for old technologies including Westergren method⁽⁵⁾. It also reduces the risk of biohazards and reduces the work load and shorten the time to deliver results^(1,6).

Rouleaux formation, which is thought to be the most important of the other stages of Red Blood Cell Sedimentation, is captured by the ISED method's quantitative photometry. Rouleaux formation occurs due to acute phase protein i.e. haptoglobin CRP and to a lesser extent due to globulin. Amongst the acute phase proteins, Fibrinogen contributes the greatest in affecting ESR⁽⁷⁾.

MATERIAL AND METHODS

A Prospective cross-sectional study conducted at Pathology Department Pak Emirates Military Hospital Rawalpindi November

2021 to April 2022. After approval from Ethics Review Committee. 100 samples incooperated in study were taken randomly from outpatient as well as inpatient department.

Samples Collection Procedure: All samples were processed within 1 hour of venipuncture. Hemolyzed samples were discarded. To separate EDTA sample tubes were taken and 3ml venous blood was collected in each tube respectively. Each sample was first analyzed by conventional Westergren method in then subjected to automated (ISED) method and results of ESR estimated by two methods were compared afterwards.

Westergren Method: 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 mark with suction applied by figure 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 the second EDTA tube automated method was applied.

ISED (ALCOR) method: It makes use of quantitative photometry to record the effects of the Rouleaux formation, an early stage of RBC sedimentation. The size of red blood cell aggregates directly relates to ESR values, which rise in accordance with the presence and intensity of inflammation, making this stage of ESR estimate the most crucial. It requires just 100µl of samples for testing in EDTA primary tube. For quality assurance reagent known as seditrol composed of stabilized human red cells suspended in a buffered fluid and preservative is used as a control tube and running simultaneously with sample tube.

Procedure:

1 "Add sample Icon" was touched on menu screen and the tube was inserted in the ISED machine.

2 The tube was oriented in such a position that barcode label should be facing towards the internal barcode reader so that the machine can recognize the barcode. The tube was rotated until the

beep was heard. The beep indicates that instrument has read the barcode successfully.

3 After 20 sec. read the results printed by ISED.

4 Similar procedure was repeated with test sample tube.

Statistical Analysis: The data were analyzed using SPSS version 23.00. The mean and standard deviation of the continuous variable were calculated. Analyses of categorical variables were done using frequency and percentage. The independent sample t-test was used for the comparison of Westergren (Manual Method) and ISED (Automated Method). Pearson correlation was used to find out the relation between the Westergren (Manual Method) and ISED (Automated Method). The p value ≤ 0.05 was considered significant.

RESULTS

200 samples in total were used for Westergren's (Manual Method) and ISED's ESR estimates (Automated Method). Mean age of the

patients was 50.74 ± 16.84 years range from 8 to 88 years, out of total 54 % patients were male and 46 % were females, 51 % patients from OPD and 49 % were admitted patients Shown in Table -1.

Table-1: Demographic Variables of ESR

Study Parameters	n (%)
Age (Years)	50.74 ± 16.84
Range (minimum – maximum)	8 - 88
Gender	
Male	54 %
Female	46 %
OPD Patients	51 %
Admitted Patients	49 %

Table-2: Mean Comparison of ESR by Westergren (Manual Method) and ISED (Automated Method) in various Group (n = 100)

ESR Values in Four Groups	Westergren (Manual Method)	ISED (Automated Method)	Mean Difference	p value	CV (%) Westergren (Manual Method)	CV (%) ISED (Automated Method)
All cases	60.88 ± 30.62	57.49 ± 33.48	3.39	0.747	50.29	58.23
Group 1 (0 – 20)	10.57 ± 6.16	10.30 ± 6.83	0.27	0.933	58.27	66.31
Group 2 (21 – 50)	34.09 ± 8.62	35.81 ± 8.85	- 1.72	0.461	25.28	24.71
Group 3 (51 – 100)	72.98 ± 12.69	74.00 ± 12.30	- 1.02	0.731	17.38	16.62
Group 4 (above 100)	115.82 ± 10.75	122.08 ± 10.82	- 6.26	0.179	9.28	8.86

In Table II, mean and standard deviation (SD), mean difference (DMD) with p values, and coefficient of variance (CV) estimated for Westergren (Manual Method) and ISED (Automated Method) in total samples and across four groups are displayed. In all groups ($p > 0.05$), there was no statistically significant difference between the two groups Westergren (Manual Method) and ISED (Automated Method).

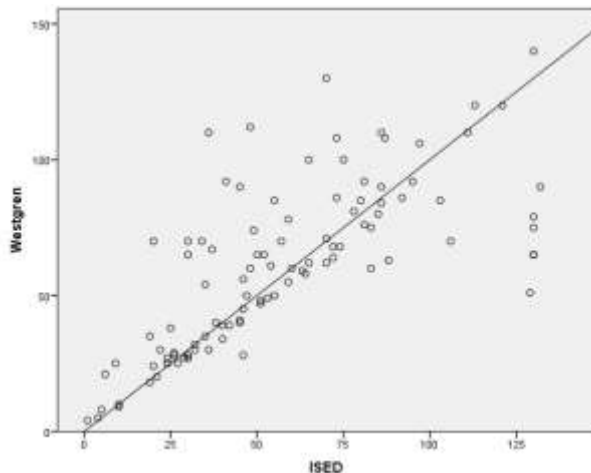


Figure 1: Pearson correlation of ESR amongst Westergren (Manual Method) and ISED (Automated Method)

There was positive correlation between Westergren and ISED Method $r = 0.715$ that showed that both methods are equally efficient and authentic but Westergren method showed the coefficient of variance 50.29% while ISED 58.23% which makes the Gold standard method more reliable and authentic comparatively.

DISCUSSION

ESR is a non-specific, affordable, and reasonably easy haematological test for identifying inflammation that may be brought on by an infection, a cancer, or an autoimmune illness. ⁽⁸⁾

The ICSH has proposed alternative methods which are considered to be as reliable as conventional methods to evaluate ESR In order to validate the results ^(8, 9). The statistical methods of bland and Altman and coefficient of correlation are frequently used to assess ESR. ^(2,3,4,10) We carried out this study to compare results of ESR carried out by Gold Standard Westergren method and automated ISED (ALCOR) method to validate the results obtained by automated method.

Our findings are entirely consistent with the research conducted by Naghmi et al. in 2017. ^{8,10,11} at Islamabad Medical and Dental Hospital 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 . Our results also coincides with the study conducted at faculty of Allied Health Sciences Kuwait by Saud M. Al Fadhil et. al, 2005 ^{4,12} which 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 as is shown by Pearson coefficient and p-value in our study. Our results are also not in agreement with the study conducted at Southampton General Hospital UK by A.C Browning et. al, 2000 ^{6,13} where there was a huge difference in results calculated by automated system and conventional manual seditainer method. Furthermore, our results are completely opposing the results found in anemic patients in Tertiary Referral Institute of North India by Vikram Narang et. al, 2019 ^{14,15} where there was clearly shown in results that automated method (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 inspite 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

CONCLUSION

The conventional Gold standard method has proven to be more efficient by CV % but p-value calculated individually as well as in

all cases was statistically insignificant $> 0.05\%$. So further studies should be carried out with more samples size to validate the finding of our study. There is propensity of automated methods to show discrepancies sporadically all together. So cannot replace conventional method.

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