

Validation of Automated ESR Methods with Conventional Method as Gold Standard

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Abstract

Objective: To compare the ESR values by Ves-Matic Easy and Vacuette SRS 20/11 with conventional Westergren method, aiming to validate the automated methods.

Material and Methods: A cross-sectional study was conducted at Islamabad Diagnostic centre, Islamabad. A total of 108 blood samples were subjected to ESR estimation by manual Westergren method and by automated (Ves-Matic and Vacuette SRS) methods. Results were analyzed on SPSS ver. 17. Results were compared and their correlation was calculated using Pearson correlation coefficient.

Results: There is strong positive correlation between Westergren method and Ves-Matic easy methods with Pearson coefficient of 0.97 and highly significant p value of 0.000. Results also show strong positive correlation between Westergren and Vacuette SRS methods with Pearson coefficient of 0.95 and highly significant p value of 0.00.

Conclusion: Both automated methods (Ves-Matic Easy and Vacuette SRS) show good correlation to manual Westergren method, and are reliable and suitable for use in high workload clinical laboratory.

Key Words: ESR, Westergren method, manual ESR, automated ESR

Introduction

The erythrocyte sedimentation rate (ESR) is a most widely used test in clinical practice. It increases in various infectious diseases, inflammations, malignancies and autoimmune diseases reflecting both the plasma (acute-phase proteins) and cellular properties (red blood cell concentration, RBC surface charge and aggregation).¹⁻⁴

These combine and cause, to a greater or lesser extent a difference in the specific gravity between red cells and plasma, and dictate the degree to which the red cells form rouleaux.

ESR is a particularly sensitive indicator of silent and chronic inflammation that is the underlying process in many diseases.^{5,6} Thus despite the availability of alternative inflammatory parameters such as CRP level and leukocyte (neutrophil) count, it is still a frequently requested parameter and, at the moment, probably the most widely measured index of acute phase response.

The method for the ESR was first described in 1921 by Dr R Fahraeus and Dr A Westergren.^{7,8} It rapidly became a common screening test worldwide for acute phase proteins and chronic diseases. There are several different methods to determine the ESR, but the conventional Westergren method is still considered as the reference method. This method determines erythrocyte sedimentation after 1 hour in a vertically mounted tube of defined length and bore size. However, it is not an automated method, and also carries a risk of infection, needs relatively large volumes of blood, and, with an analysis time of 1 hour, is time-consuming.⁹ Increased awareness of biohazards risk to laboratory staff has led to safer methods for performing the ESR such as vacuum controlled aspiration of sample and automated mixing of sample with sodium citrate anticoagulant present in the tube.

To overcome the practical drawbacks of the original Westergren ESR method, several methods were introduced. These methods measure the ESR in dedicated tubes using whole blood diluted with EDTA or citrate. Sedimentation (in mms.) of erythrocytes is recorded and subsequently recalculated to Westergren units (mm/h). The advantage of these methods over a manual Westergren-based method is that they provide a fully closed, automated system with results that are more readily available. As regards Ves-Matic

and Vacuette SRS methods, these show good correlation with the conventional Westergren method.^{10,11}

The Ves-Matic method uses standard blood sample tubes for direct measurement of ESR.

This method has the advantages of automated mixing of blood sample, shortened testing time (20 minutes), and automated end-point reading by a digital sensor. The Vacuette SRS 20/11 method reads the result after 30 minutes. These automated methods show good correlation with the conventional Westergren reference method.

This study was designed to compare the ESR values by Ves-Matic Easy Vacuette SRS 20/11 and with conventional Westergren method, aiming to validate the automated methods.

Material and Methods

A cross-sectional study was conducted at Islamabad Diagnostic Centre, Islamabad from April to May 2012. In total 108 samples were subjected to ESR estimation by Westergren, Ves-Matic Easy and Vacuette SRS 20/11 methods. For all three methods, blood was diluted with citrate solution.

The Ves-Matic Easy is a bench instrument designed and programmed to determine the ESR in a maximum of 10 samples of blood contained in dedicated cuvettes (which contain sodium citrate as an anticoagulant), simultaneously or individually in random access mode. The instrument is controlled by a microprocessor. The cuvettes after being filled with 1.1ml carefully mixed blood to an intended height of 60 mm, are placed in the instrument. The instrument maintains the cuvettes at an angle 18° to the vertical and a photoelectric cell then passes up the outside of each cuvette to record the height of the column of red cells at which light transmission occurs. After 20 minutes of sedimentation, timed electronically, the new level at which light passes through the column is recorded and the decrease in height is corrected mathematically to give a result which is stated to be comparable with the Westergren ESR at one hour. To determine the accuracy and precision of the results, quality control material provided by the manufacturer was used.

The Vacuette SRS 20/11 functioning is almost the same as Ves-matic Easy, but it holds 20 samples at one time, and its measuring time is 30 minutes. The Westergren ESR was performed according to the standardized selected method of the International Council for standardization for Hematology (ICSH) using sodium citrate as an anticoagulant.

Results were entered on SPSS version 17 for analysis. We divided our patients into three groups on the basis of ESR values obtained by Westergren method: Group1: ESR 0-20; Group2: ESR 21-50; Group 3: ESR 51-100. Means of results obtained from manual and automated methods were compared in all samples and in three groups and their p values were calculated; p Value of ≤0.05 was taken as statistically significant. Coefficient of variance was

calculated for all methods. Pearson correlation was calculated for both Ves-Matic and Vacuette SRS Methods.

Results

Table 1 shows mean and SD values, difference of mean with p value and coefficient of variance (CV) calculated for Westergren method, Ves-Matic easy and Vacuette SRS methods, in total samples and among three groups. As shown in the table CV of Ves-Matic method is lower pointing towards more reliability of this method. Table 1 also shows the means and difference in means calculated for both the methods in comparison to that of Westergren method. As shown in the table the difference of means is insignificant with Ves-Matic method at higher ESR values. However with Vacuette SRS method this difference was significant with Vacuette SRS method in group 2 (ESR 21-50 mm/hr)

Table 1: Evaluation of results of various groups by manual and automated methods (n 108)

Methods	Mean ± SD (mm/time)	Difference of Means	p value	CV (%)
All Cases (n 108)				
Westergren	22.96 ± 21.29	-	-	92.72
Vest Matic	21.86 ± 21.27	1.1	0.02	97.30
Vacuette SRS	20.36 ± 19.96	2.6	0.00	98.03
Group 1 (n 65)				
Westergren	8.98 ± 5.33	-		59.39
Vest Matic	8.28 ± 4.64	0.7	0.02	56.02
Vacuette SRS	8.52 ± 4.98	0.46	0.19	58.47
Group 2 (n 29)				
Westergren	32.90 ± 7.97	-		24.22
Vest Matic	30.00 ± 8.22	2.89	0.06	27.38
Vacuette SRS	26.28 ± 8.89	6.62	0.00	33.05
Group 3 (n 14)				
Westergren	67.29 ± 11.86	-		17.63
Vest Matic	68.07 ± 10.49	-0.78	0.54	14.90
Vacuette SRS	63.08 ± 15.90	4.21	0.14	25.21

Significant p value: ≤ 0.05

Table 2: Pearson correlation among different methods (n 108)

Methods	Correlation*	p value
Westergren and Vest Matic Methods	r = 0.97	0.00
Westergren and Vacuette SRS Methods	r = 0.95	0.00

**Correlation is significant at the 0.01

Table 2 shows the Pearson correlation among different methods. As is shown in the table there is strong positive correlation between Westergren method and Ves-Matic easy methods with Pearson coefficient of 0.97 and highly significant p value of 0.000. Results also show strong positive correlation between Westergren and Vacuette SRS methods of 0.95 with highly significant p value of 0.00. Figures 1 and 2 show that there is significant linear

correlation between Westergren and Ves-Matic methods and Westergren and Vacuette SRS methods

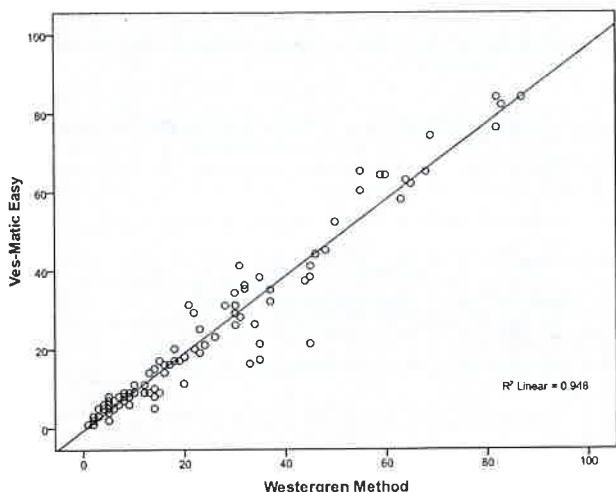


Figure 1: Correlation of ESR Estimation (in mm/hr) by Vest Matic method and Westergren method

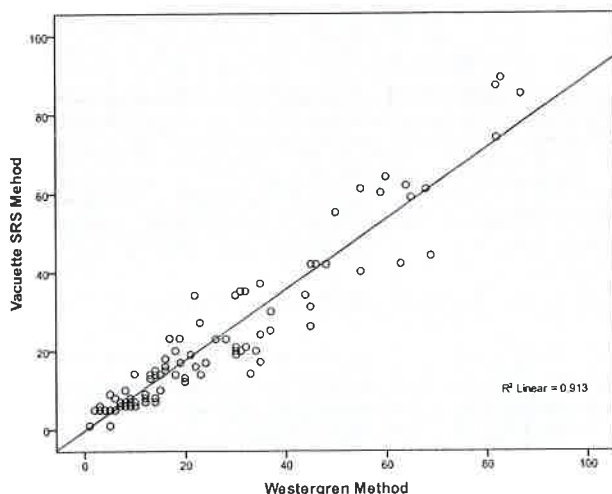


Figure 2: Correlation of ESR Estimation (in mm/hr) by Vacuette SRS method and Westergren method

Discussion

The erythrocyte sedimentation rate is a relatively simple and inexpensive test used to assess patients with acute or chronic inflammatory processes.^{12,13} It serves as a useful aid in the diagnosis of various clinical conditions, and has been shown to correlate with an unfavourable prognosis in neoplastic diseases and coronary artery disease.¹⁴⁻¹⁷

In recognition of the need for a standardization of the measurement of ESR, the ICSH has proposed a protocol for the evaluation of alternative methodologies against the reference method has also been proposed: The new technologies must be tested over a range of ESR values of 2–120 mm. In this comparison, 95% of the differences

should be 5 mm or less, with larger differences associated with higher ESR values. The statistical methods recommended for ESR evaluations are the coefficient of correlation, the Passing-Bablok regression and the Bland-Altman statistical method.¹⁸

We carried out this study to look for correlation of two automated methods (Ves-Matic and Vacuette SRS) with Westergren method. We found strong positive correlation of both the methods with Westergren method with highly significant p-value of 0.000. We also calculated Coefficient of variance for different methods and it was found to be lower in Ves-Matic method indicating more reliability of this method. Other studies also confirmed the excellent correlation between Ves-Matic and Westergren^{19,20}

There are some other automated methods which are in use. Horsti J carried out study with StaRRsed and reported that Starr-sed has advantages, as it offers savings of consumables, safety and fluent workflow. StaRRsed has many excellent technical properties and the study proved a fairly good correlation between two methods ($R^2 = 0.72$) and found that StaRRsed is in better agreement with the Westergren method. They reported that differences between StaRRsed and classic Westergren method that were observed in their study were, in many cases, acceptable and clinically insignificant.²¹ Fiorucci also compared Test 1 system with Westergren method but results showed a lower degree of agreement between these two methods. They proposed though the results obtained with Test 1 system are within acceptable limits, the Ves-Matic instrument shows better correlation with the Westergren method, with a consequently lower risk of false positive and false negative results.²² However Curvers et al reported less correlation of Ves-Matic with Westergren method and good correlation of SEDI system and StaRRsed methods with conventional method.²³

In our study mean difference of three methods in three groups has shown that lower values were recorded by Vacuette SRS method as compared to Westergren method particularly at higher ESR values. However with Ves-Matic method this difference is less than three even at higher ESR values. Subramanian A. et al recommended that a correction factor be applied for the range of ESR values with such discrepancies.²⁴ There were only 14 samples in group 3 (ESR 51-100 mm/hr) and 29 sample in group 2 (ESR 21-50 mm/hr). Since these two groups are most important as they comprise the cases with ESR values indicating pathological basis. Our recommendation is to carry out further studies with at-least 50 samples in each group and the third group should also contain samples with ESR more than 100 according to Westergren method. However with current available data both the methods show good correlation with Westergren method.

In conclusion, both automated method (Ves-Matic Easy and Vacuette SRS) show good correlation to Westergren method (as shown by Pearson correlation coefficient). These findings indicate that Ves-Matic Easy and Vacuette SRS are

reliable and suitable system for high workload clinical laboratory. The Ves-Matic Easy method however shows a better correlation with less difference in means, particularly at higher ESR values.

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