Comparison of Erythrocyte Sedimentation Rate Measurement by the Automated SEDIsystem™ and Conventional Westergren Method Using the Bland and Altman Statistical Method

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Introduction

Although erythrocyte sedimentation rate (ESR) is not considered a specific diagnostic test, it is used in monitoring certain groups of patients such as those with rheumatoid arthritis, temporal arthritis, polymyalgia rheumatica and Hodgkin’s disease, where disease activity is mirrored by changes in the ESR. The most satisfactory method of performing ESR was introduced by Westergren in 1921 [1]. This method was recommended by the International Council for Standardization in Haematology [2] and the National Committee for Clinical Laboratory Standards [3].

Automation of ESR measurement could minimize the biological risks, reduce the amount of the blood taken for the test and the time required to release the results. Of the available automated systems for ESR measurement, the SEDIsystem™ (Becton Dickinson, Vacutainer Systems) showed a wide degree of scatter between results obtained by the two ESR techniques that was not clearly demonstrated using the linear regression analysis. The automated system was found to underestimate ESR with the Bland and Altman statistical analysis, and therefore a correction factor is recommended.

Objectives:
To compare the performance of SEDIsystem™, a fully automated analyzer for the measurement of the erythrocyte sedimentation rate (ESR), with the manual Westergren method.

Materials and Methods:
Both methods were applied to 150 randomly selected subjects. The linear regression and Bland and Altman data analysis methods were used to measure the agreement between the automated and manual methods.

Results:
The regression analysis showed a good correlation between the two methods ($r = 0.91$). The Bland and Altman data analysis showed no systematic bias (95% confidence interval for mean difference); however, limits of agreement were between 11.52 and −37.88. This indicates that ESR values measured by the SEDIsystem may be 11.52 mm/h above or 37.88 mm/h below the reference method. A greater scatter of data was also observed with abnormally high (>25 mm/h) ESR results (mean of difference = −21.4 and limits of agreement = −45.2 and 2.26) compared with normal (<25 mm/h) readings (mean of difference = −3.9 and limits of agreement = −13.5 and 5.7).

Conclusion:
The Bland and Altman statistical analysis showed a wide degree of scatter between results obtained by the two ESR techniques that was not clearly demonstrated using the linear regression analysis. The automated system was found to underestimate ESR with the Bland and Altman statistical analysis, and therefore a correction factor is recommended.
is designed to give ESR results in Westergren-equivalent values. It comprises vacuum sampling tubes (SEDITAINER® 1.8 tubes) and an automatic reader.

In this study, we compared the automated SEDIsystem for ESR determination with the conventional manual Westergren method using statistical analysis according to Bland and Altman [4].

Material and Methods

Samples
One hundred and fifty blood samples were collected; each sample was placed into two tubes – a K3-EDTA Becton Dickinson vacuum collection tube and a SEDITAINER® 1.8 tube. The blood samples were collected from the outpatient department at a major hospital in Kuwait. Subjects coming for routine checkup or follow-up were randomly chosen. All blood samples were assayed within 1 h of venipuncture. Hemolyzed samples were excluded. Each sample was analyzed once with the Westergren method (the standard method) and three times with the SEDIsystem (the test method); the average of the three automated runs was calculated and compared with the results of the Westergren method.

The Westergren Method
The Westergren method employs a 300-mm vertically aligned column, 2.55 mm in diameter and open at each end. K3-EDTA anticoagulated venous blood diluted 4:1 in citrate was transferred into the column reaching 200 mm from the bottom of the tube. The distance that the column of blood falls in 1 h is recorded and reported in mm/h.

The SEDIsystem
SEDITAINER® vacuum collection glass tubes (1.8 ml, 100 mm long, 6 mm wide) were filled with approximately 1.8 ml of blood. The tubes contain 0.452 ml of 0.105 M citrate buffer solution as an anticoagulant. The blood/citrate mix reaches 80 ml from the bottom of the column. The sedimentation reading is taken through a 47-mm-high window starting at the lower edge of the stopper. The procedure of this test is to distribute the 1.8-ml tubes on three racks, each carrying 15 tubes. The samples are homogenized, passed through a CCD mobile camera, and the ESR is recorded after 20 min. A conversion scale supplied with the racks in the SEDIsystem is used to give results in Westergren-equivalent values.

Statistical Analysis
SPSS for Windows, version 120 (SPSS, Inc., Chicago, Ill., USA), was used for data analysis. Pearson’s regression analysis was used to analyze the correlation coefficient (r) between the reference and the new method. Since a high correlation does not prove the agreement between the two measurements, the Bland and Altman statistical method was used to measure the limits of agreement of the two measurements [4]. The difference (test method – standard method) between the two measurements was calculated and plotted against the mean of the two measurements. Then the limits of agreement were calculated as d ± 2 SD where ‘d’ is the mean of the difference between the two measurements and ‘SD’ is the standard deviation of the difference.

Results
Out of the 150 samples analyzed and for both reference methods, 69 were within the ESR reference range used in the hospital (0–25 mm/h), while 81 were abnormally high (>25 mm/h).

Pearson’s regression analysis between the values of the reference method and the mean of the three runs of the new method showed a good correlation (r = 0.91, p < 0.0001) (fig. 1). However, it is worth noting that r measures a relation between two variables, not the agreement between them. A perfect agreement will only be found if all the points in figure 1 lie along the line of equality, but a perfect correlation is found if the points lie along any straight line. Therefore, the calculation of r is not sufficient and could be misleading since the strong correlation does not show the agreement between the two measurements.

The calculated mean of the differences between the two methods was –13.18 mm/h, where zero is the predicted value if the two methods are identical in their measurements for the same sample. The limits of agreement between the two methods were 11.52 and –37.88 (fig. 2). This means that ESR values measured using the SEDIsystem may be 11.52 mm/h above or 37.88 mm/h below
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the reference method, which would be unacceptable for clinical purposes. When calculating the correlation (r) between the difference and the mean of the two methods, it was found to be 0.78. This value indicated that there was a marked difference in the variability between the two methods. The discrepancy was particularly evident in samples with high ESR values (25 mm/h). Thus, for samples with ESR readings \( \geq 25 \text{ mm/h} \), the mean of difference (–21.4) and the limits of agreement (–45.2 and 2.26) were markedly different from the corresponding values (–3.9; –13.5 and 5.7, respectively) for samples with ESR values under 25 mm/h. It is apparent from these results that samples with high ESR values varied considerably around the mean difference compared with samples that had normal ESR readings.

**Discussion**

Although the conventional Westergren method for ESR remains the gold standard for measuring ESR, it is laborious, time consuming and involves high biohazard risks. There is, therefore, a need to establish systems that would overcome these drawbacks. Several automated closed systems are now available, e.g. SEDIsystem (Becton Dickinson), Sedimatic (AnalyInstrument), Starrsed (R&R Mechatronics), Test I System (SIRE) and others [6]. These systems were shown to provide fast, simple and biologically safe means of measuring ESR.

Despite these advantages, it is important to validate these automatic techniques against the commonly used manual Westergren method. In the present study, the results obtained with the two ESR measuring methods showed a strong correlation when the results were analyzed using simple Pearson’s regression analysis. The Bland and Altman plot (fig. 2), on the other hand, displayed a considerable lack of agreement between the two ESR methods, with discrepancies of up to 38 mm/h. Our findings are similar to those from previous reports [7–11], where a strong correlation exists between the two ESR methods using Pearson’s regression analysis but not with the Bland and Altman statistical approach. However, it is widely accepted that the Bland and Altman approach is more accurate than the regression analysis [7, 12, 13].

The present study showed that the automated test system tended to underestimate (mean negative bias – 13.1 mm/h) ESR values. In fact, out of the 81 samples with abnormally high ESR, 38 were underestimated by the automated system, and to a clinically significant degree. In contrast, this discrepancy was not found when samples with normal ESR were analyzed.

**Conclusions**

Our study demonstrates that the automated system tends to underestimate ESR, sometimes to a degree that is clinically significant. Therefore, if the SEDIsystem is to replace the Westergren manual ESR method, a correction factor that will correct for the underestimation must be used. Alternatively, a SEDIsystem reference range should be established.

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References


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