# Erythrocyte Sedimentation Rate Measurement by VES Matic Cube 80 in Relation to Inflammation Plasma Proteins

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Westergren method is considered as the reference procedure to measure Erythrocyte Sedimentation Rate (ESR) by the International Council for Standardization in Haematology. However, a closed automated method, VES Matic Cube 80 (DIESSE S.p.A., Siena, Italy), has been introduced as a new ESR measurement instrument. In this article, we report two different studies: first, we compared the two methods (Westergren and VES Matic Cube 80) and second, we correlated the inflammatory state of 248 patients with their ESR values. Total protein, albumin, C-reactive protein, and other inflammatory proteins were detected in each sample. The results obtained using VES Matic Cube 80 demonstrated a good correlation with those obtained using the Westergren method (Ordinary linear regression: $y = 0.955x - 0.205$, $r^2 = 0.816$, $P < 0.05$; Passing–Bablock regression equation: $y = 0.9153x - 0.5763$; Bland–Altman analysis: bias 1.2; limits of agreement $-17.4 - 19.9$) and with the inflammatory protein levels (CRP: $r = 0.554$ and $r = 0.498$ and Fibrinogen: $r = 0.699$ and $r = 0.663$ for Ves Matic Cube 80 and Westergren, respectively), supporting the hypothesis that VES Matic Cube 80 offers a fast and safe ESR determination, ensuring precision and a very good correlation with the reference method. J. Clin. Lab. Anal. 25:198–202, 2011. © 2011 Wiley-Liss, Inc.

**Key words:** erythrocyte sedimentation rate; automated laboratory instrument; VES Matic Cube 80; Westergren method; inflammation

## INTRODUCTION

The erythrocyte sedimentation rate (ESR) is widely used as a screening test for patients with acute and chronic inflammatory diseases. Although it is not considered a specific diagnostic test, it is used in monitoring and follow-up of certain groups of patients, such as those with rheumatoid arthritis, temporal arthritis, polymyalgia rheumatic, and Hodgkin's disease, where disease activity is mirrored by changes in the ESR (1,2). Recently, it was reported to be a prognostic value in the case of acute coronary syndrome and stroke and an independent predictor of mortality (3).

The International Council Standardization in Haematology (ICSH) (4) and the National Committee for Clinical Laboratory Standards (5) selected the Westergren method (6), which makes use of undiluted blood sample with $K_2EDTA$ as anticoagulant (dilution less than 1%), as the reference technique for measuring ESR.

The original ICSH reference method (4) was based on the methodology of Fåhraeus (7) and Westergren (6), which used diluted blood (4 volumes blood plus 1 volume citrate). Modifications of these specifications, particularly the use of undiluted blood, are now recommended as the basis of a new ICSH reference method. Because this method presents a lot of variables, such as specimen collection, time, and temperature of specimen storage, several new techniques for measuring ESR have been developed and introduced in clinical laboratories.

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Recently, a closed automated ESR measurement instrument, VES Matic Cube 80 (Diesse, Diagnostica Senese S.p.A., Monteriggioni, Siena, Italy), measures the ESR directly in $K_2EDTA$ or $K_3EDTA$ blood cells counting tubes, in the absence of refluents and aerosol using optoelectronic elements, characterized with white light, high power LED, and analogic photo sensor.

The instrument can express results as EDTA or citrate sedimentation. In fact, using a special software algorithm, the system is able to transform the EDTA values as obtained in citrate (expected results).

Considering that the Westergren reference method used is based on the use of undiluted blood and that previous works (3) have already shown results in citrate, in this study we considered only EDTA blood outcome. To confirm system accuracy, a very careful comparison of this technique with the reference method is required (4,5).

For this reason, the aim of this work is to measure the ESR value of $K_2EDTA$ blood samples from randomly chosen patients using VES Matic Cube 80 and to compare the results with Westergren method.

Moreover, in response to inflammatory states, there is an increment in the levels of some acute phase proteins, such as C-reactive protein (CRP), fibrinogen, immunoglobulin, and other important serum proteins, involved in erythrocyte rouleaux formation.

Consequently, we also applied the two methods to asses their ability to evaluate inflammatory states, determining the correlation between CRP, fibrinogen, albumin and α1-globulin, and the ESR values.

## MATERIALS AND METHODS

### Subjects

The study subjects were chosen randomly from the entire population of hospitalized and ambulatory patients to which ESR determination was routinely prescribed. All blood samples were collected in 3 ml tubes (Vacutainer, Becton Dickinson, UK) with the $K_2EDTA$ anticoagulant (1.8 mg/ml) under standard conditions and tested within 4 hr of venipuncture, according to ICSH recommendations.

The population consisted of 248 patient samples obtained at the Santa Maria alle Scotte Hospital, Siena, Italy, and analyzed there. The test group was composed of 106 males and 142 females, both having a mean age of 58.

### Westergren Method

The reference method was performed according to ICSH specifications on undiluted blood samples anticoagulated with $K_2EDTA$ using glass pipettes.

Test tubes were gently and manually mixed for 20 times and pipettes were inserted into tubes until the column of blood rose to the start stopper. During sedimentation, the graduated pipettes were mounted vertically on appropriate supporting racks and kept at a maximum temperature of 25°C. After 1 hr, RBC sedimentation was recorded through visual determination.

### VES Matic Cube 80 Method

VES Matic Cube 80 (Diesse Diagnostica Senese S.p.A., Italy) is a closed automatic system for determining ESR in $K_2EDTA$ or $K_3EDTA$ tubes. The entire test is performed in the total absence of refluents and aerosol, produced by eventual perforation of the caps from samples to be tested.

Venous samples were inserted into specific analyzer's rack and transferred to the test tube holder chain, passing mixing part and transported to the reader point 1. The speed of the chain movement is controlled to allow the samples to settle for a period of 20 min before the final reading at reader point 2.

All the phases of the ESR are measured thanks to an innovative optical system by using optoelectronic elements (white light, high power LED, and analogic photo sensor).

### Measurement of Inflammatory Plasma Proteins

Plasma proteins were measured at Santa Maria alle Scotte Hospital, Siena, Italy. The $\alpha_1$-globulins were measured by capillary electrophoresis. Fibrinogen was determined calculating the prothrombin time (BCS XP, Siemens, Munich, Germany), CRP and albumin fractions were measured with an immunoturbidimetric assay (COBAS 6000, Roche, Basel, Switzerland).

### Statistical Analysis

Each sample was analyzed in duplicate, both for manual and automated methods, as suggested by the EP09-A2 guideline (8) which was only been partially applied.

Data are reported as mean±standard deviation (SD) and a paired student $t$-test was used to compare the means.

Nonparametric test of Spearman was used to study correlation (ρ, correlation factor).

Method comparison was performed by ordinary linear regression (OLR), Passing and Bablock regression (9) and Bland–Altman analysis (10), comparing VES Matic Cube 80 data and those of the Westergren method.

ESR data and plasma proteins levels were analyzed by correlations study ($r$).

All statistical calculations were performed using Microsoft Excel 2007 software (Microsoft, Seattle, WA) and MedCalc software, Version 10.1.5.0 (Mariekerke,

Belgium). Values of $P < 0.05$ were considered statistically significant.

## RESULTS

### Comparison Between Westergren and VES Matic Cube 80 Methods

ESR was measured in 248 blood samples by Westergren and VES Matic Cube 80 methods. The mean±SD ESR was 23.1±20.9 mm/hr (range: 1–110 mm/hr) for the reference method and 21.86±22.1 mm/hr (range: 1–90 mm/hr) for VES Matic Cube 80.

There was a significant correlation between Westergren and VES Matic Cube 80 measurements ($y = 0.955x - 0.205$, $r^2 = 0.816$, $P < 0.05$) (Fig. 1). The same results were also obtained considering the Spearman's rank correlation coefficient ($\rho$), which presents a value of 0.951 (95% CI: 0.937–0.961) with a $P < 0.0001$ (Fig. 1).

Also Passing and Bablock (scatter and residual plots) showed a good correlation between the methods (Fig. 2). As shown in Table 1, the linear regression equation, $y = 0.9153x - 0.5763$, presented good slope and intercept.

The agreement between the results obtained by different methods was also demonstrated by Bland–Altman analysis (Fig. 3). It showed a positive mean bias, equal to 1.2 mm/hr (limits of agreement, −17.4–19.9), indicating that the test method values were slightly lower than the Westergren ones.

### Correlation Between Westergren and VES Matic Cube 80 Methods With Levels of Some Plasma Proteins

The assessment of the inflammatory state has been made with a rather peculiar approach. Obviously,
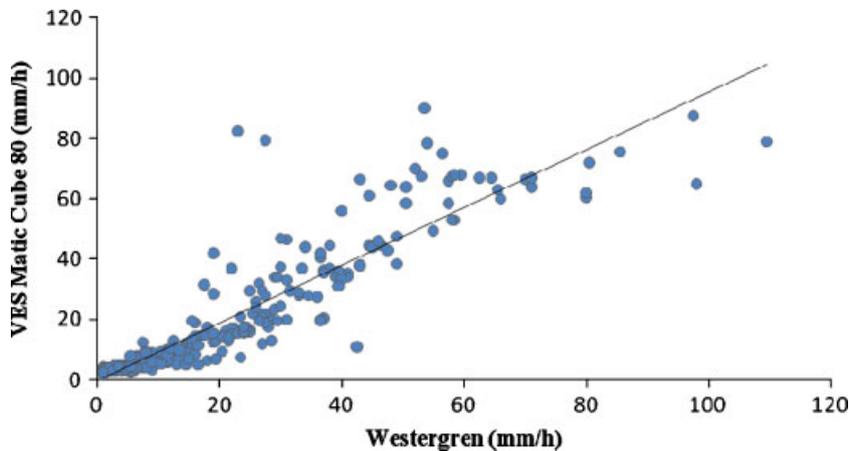
**Fig. 2.** Comparison of Westergren and VES Matic Cube 80 methods for ESR measurements: Passing and Bablok analysis. (**A**) Scatter plot of correlation between the methods. (**B**) Residual plot.



**Fig. 1.** Comparison of Westergren and VES Matic Cube 80 methods for ESR measurements. Ordinary linear regression of correlation between the methods ($y = 0.955x - 0.205$, $r^2 = 0.816$, $P < 0.05$).
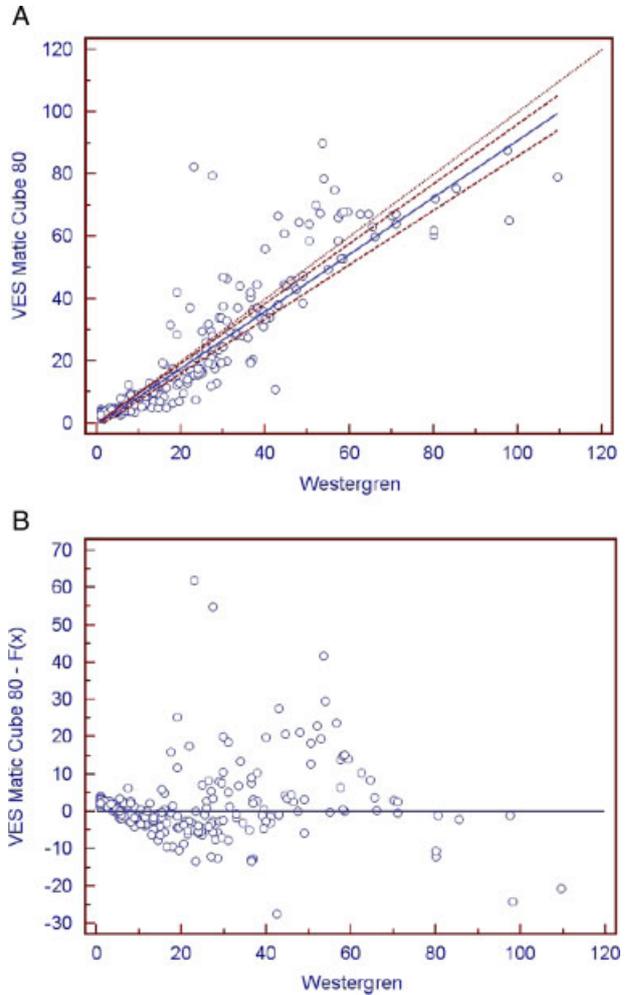
among all analyzed proteins, only a few of them were dealing with acute phase response.

Analyzed samples demonstrated that there were significant correlations (Colton, 1974) (11) between fibrinogen, albumin, and $\alpha_1$-globulin levels and ESR for Westergren method ($P < 0.0001$) and between CRP, fibrinogen, albumin, and $\alpha_1$-globulin measurements and VES Matic Cube 80 results ($P < 0.0001$) (Table 2).

**TABLE 1. Linear Regression Analysis According to Passing and Bablok**

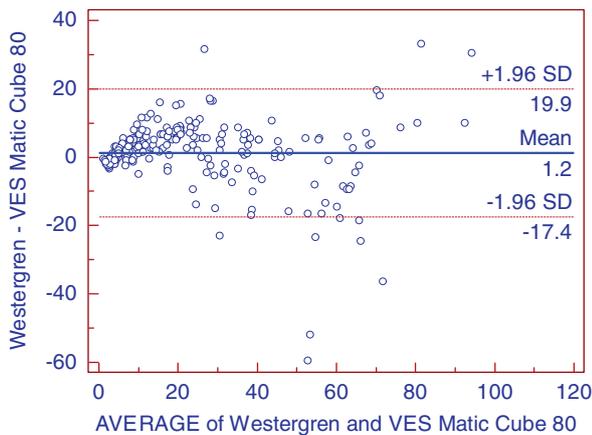| | 95% CI | |
|---|---|---|
| Regression equation | Slope B | Intercept A |
| $y = 0.9153x - 0.5763$ | 0.8696–0.9592 | −1.2551–0.1848 |



**Fig. 3.** Comparison of Westergren and VES Matic Cube 80 methods for ESR measurements. Bland–Altman plot: plot of the differences between ESR measurements ($y$-axis) and average ESR values ($x$-axis).

## CONCLUSION

Erythrocyte sedimentation represents a nonspecific reaction, which is largely used to investigate the severity of pathological processes.

ESR is not the measure of an analyte but the measure of a physical phenomenon, which depends on a large number of variables, such as dilution of blood with anticoagulants, number and size of erythrocytes, plasma density, protein concentration, internal diameter and length of the column, temperature, and time from venipuncture.

In order to reduce the importance of these variables, several new ESR measurement methods have improved the technical aspects of the testing procedure. In this regard, there are a lot of automated and semi-automated instruments able to reduce the biological risk and the testing time for example VES Matic Cube 80 (Diesse, Diagnostica Senese S.p.A.).

The results obtained by VES Matic Cube 80 analyzer showed good correlation with Westergren measurements ($\rho = 0.951$).

Furthermore, both OLR and linear regression, according to Passing and Bablok, demonstrate the high compatibility between the results obtained by VES Matic Cube 80 and those obtained by reference method.

The trueness is reflected in a low positive bias (1.2 mm/hr, Bland–Altman analysis), indicating that VES Matic Cube 80 values are a little lower than those measured using the Westergren method.

The process of erythrocyte rouleaux formation depends on the concentrations of acute phase proteins and, to a lesser degree, of the globulins (12).

Considering the protein fractions, this study demonstrates that they show a good correlation with both methods, and that the VES Matic Cube 80 system shows higher correlation coefficients ($r$) and lower $P$ values than the Westergren method.

**TABLE 2. (a) Correlation Between Westergren ESR Values and Concentration of Inflammatory Plasma Proteins in Patient Samples and (b) Correlation Between VES Matic Cube 80 ESR Values and Concentration of Inflammatory Plasma Proteins in Patient Samples**

| | Sample size | Coefficient $r$* | Significant level | 95% confidence interval for $r$ |
|---|---|---|---|---|
| (a) Correlation (Westergren—proteins) | | | | |
| Albumin | 242 | −0.5520 | $P < 0.0001$ | −0.6340–−0.4578 |
| CRP | 248 | 0.4981 | | 0.3982–0.5863 |
| Fibrinogen | 202 | 0.6634 | $P < 0.0001$ | 0.5783–0.7342 |
| $\alpha_1$-Globulin (g/dl) | 242 | 0.6021 | $P < 0.0001$ | 0.5151–0.6768 |
| (b) Correlation (VES matic cube 80—proteins) | | | | |
| Albumin | 242 | −0.5846 | $P < 0.0001$ | −0.6619–−0.4950 |
| CRP | 248 | 0.5538 | $P < 0.0001$ | 0.4611–0.6346 |
| Fibrinogen | 202 | 0.6997 | $P < 0.0001$ | 0.6217–0.7639 |
| $\alpha_1$-Globulin (g/dl) | 242 | 0.6377 | $P < 0.0001$ | 0.5564–0.7070 |

*Coefficient $r$: values standing from 0.51 to 0.75 correspond to a moderate/good correlation (11)

Considering Fibrinogen and CRP, the most important acute phase proteins, which present the greatest impact on ESR, our results demonstrate that their serum concentration shows higher correlation coefficients ($r$) with the ESR values obtained using VES Matic Cube 80 rather than those obtained by the Westergren reference method (0.6997 and 0.6634; 0.5538 and 0.4981, respectively).

We demonstrate that the VES Matic Cube 80 values correlate better with inflammatory plasma proteins concentration rather than the Westergren ESR data.

As demonstrated by our findings, we can conclude that VES Matic Cube 80 offers a fast, reliable, standardized, simplified, and safe determination of ESR, ensuring a very good correlation with the Westergren method.

Moreover, comparing test and reference method, VES Matic Cube 80 measurements shows the better presence of inflammation in patients with acute and chronic inflammatory diseases.

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