Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergren-based methods

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Evaluation of the Ves-Matic Cube 200 Erythrocyte Sedimentation Method

Comparison With Westergren-Based Methods

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Key Words: Erythrocyte sedimentation rate; ESR; Ves-Matic Cube 200; Westergren; SEDIsystem; StaRRsed; hematocrit

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Abstract

The erythrocyte sedimentation rate (ESR) is still a widely used parameter for acute phase inflammation. Recently, new methods based on direct undiluted measurement of ESR in a standard EDTA tube have been developed. We evaluated the analytic performance of one of these new methods, the Ves-Matic Cube 200 (Diesse Diagnostica Senese, Siena, Italy), and compared it with several established Westergren-based diluted methods. The Ves-Matic Cube 200 showed a poor correlation \( r = 0.83 \) with the International Council for Standardization in Haematology Westergren reference method, mainly caused by a considerable negative bias at low ESR levels. Moreover, a random bias was found at higher ESR levels that correlated with hematocrit levels, suggesting a differential influence of packed cell volume on the Ves-Matic Cube 200 results compared with Westergren results. We conclude that the Ves-Matic Cube 200 method is not interchangeable with Westergren-based diluted methods and generates ESR results that are too deviant to be clinically acceptable.

The erythrocyte sedimentation rate (ESR) is a relatively simple and inexpensive test used to assess the acute phase response in inflammation. Although measurement of ESR is sometimes referred to as redundant, it is still a frequently requested diagnostic parameter. The ESR is generally determined by several parameters, including size, shape, and number of RBCs, fibrinogen concentration, globulin concentration, and temperature. Despite being nonspecific, the measurement of ESR can be informative in the diagnosis and follow-up of infection or inflammatory diseases such as rheumatoid arthritis, giant cell arteritis, and polymyalgia rheumatica. Indeed, polymyalgia rheumatica and giant cell arteritis are frequently accompanied by markedly elevated levels of the ESR (often higher than 100 mm/h). Also, the ESR may be a useful marker of lupus activity and a predictor of organ damage, although normal levels do not rule out underlying diseases.

There are several different methods to determine the ESR, but the conventional Westergren method is still referred to as the reference method for measurement of ESR and for validation of new ESR methods. This 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. For practical reasons, the Westergren method itself is sparsely used in the routine determination of the ESR. It carries a risk of infection (open tubes), needs relatively large volumes of blood, and, with an analysis time of 1 hour, is time-consuming.1

To overcome the practical drawbacks of the original Westergren ESR method, several methods based on...
the conventional Westergren method were introduced. The StaRRsed (InteRRliner, Mechatronics, Zwaag, the Netherlands) and the SEDIsystem (Becton Dickinson, Leiden, the Netherlands) are examples of these automated or semiautomated ESR methods. In accordance with the conventional Westergren method, these methods measure the ESR in dedicated tubes using whole blood diluted with citrate. Sedimentation (in millimeters) 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. Moreover, previous studies have shown that both methods show good correlation with the conventional Westergren method.2-4

In recent years, technological advances have resulted in the introduction of novel methods that measure the ESR directly from a standard EDTA-anticoagulated tube. One of these methods is the Test 1 system (Alifax, Padova, Italy), which performs ESR measurement by using a small volume of undiluted EDTA-anticoagulated blood by a microcentrifugation method. This method reduces the analytic time to several minutes and avoids the need for an additional blood sample. Although this method shows good correlation with conventional Westergren and Westergren-based methods,4,5 it was shown recently that Test 1 is less influenced by the presence of monoclonal proteins than Westergren-based methods6 and may, in fact, measure only RBC aggregation rather than the entire sedimentation process as determined by the Westergren reference method.7

In this report, we present the validation of a novel ESR analyzer, the Ves-Matic Cube 200 (Diesse Diagnostica Senese, Siena, Italy), that measures ESR directly from a standard capped EDTA blood sample tube. Unlike the Test 1 ESR method, the Ves-Matic Cube 200 allows the sample to settle for a period of 20 minutes before final optical reading, thereby analyzing all 3 phases (aggregation, sedimentation, and packing) of erythrocyte sedimentation.

To compare analytic performances of the Ves-Matic Cube 200 and Westergren-based citrated methods, we measured ESR in samples from 244 randomly selected hospitalized patients and patients of general practitioners. Sedimentation rates measured with the Ves-Matic Cube 200 analyzer were compared with results obtained by the Westergren-based StaRRsed or SEDIsystem methods and the International Council for Standardization in Haematology (ICSH) Westergren reference method. Moreover, analytic precision profiles of ESR methods used were determined, and, if necessary, deviant results were analyzed to identify variables that could differentially influence ESR results measured by the Ves-Matic Cube 200.

Materials and Methods

Patients and Blood Samples

ESRs were assessed in 244 randomly selected patient samples. Samples from hospitalized patients in 2 centers (Spaarne Ziekenhuis, Hoofddorp, the Netherlands, and Catharina-ziekenhuis, Eindhoven, the Netherlands) and patients of general practitioners located near these hospitals were obtained. Citrated (Seditainer ESR tubes, Becton Dickinson) or EDTA-anticoagulated (in Vacutainer tubes, Becton Dickinson) whole blood was used to assess the ESR within 4 hours after blood drawing. At one center (Hoofddorp), 101 citrated blood samples were measured using the SEDIsystem, and separately, 101 EDTA-anticoagulated blood samples were used to assess sedimentation in the Ves-Matic Cube 200 system and by the manual Westergren method. At the other center (Eindhoven) in 143 samples, StaRRsed ESR was first performed, after which the ESR was measured with the Ves-Matic Cube 200 system. Subsequently, when enough whole blood was available, the manual Westergren was performed.

ESR System Descriptions

Measurement of ESR in the SEDI system is performed by placing the blood-filled Seditainer ESR tubes in a system rack. The samples are homogenized automatically, after which the tubes pass a charge-coupled device camera that measures the initial cell layer height followed by final sedimentation level reading after 20 minutes. Finally, the system converts the measurement to generate results that correlate with the conventional Westergren method.

ESR measurement in the StaRRsed is performed by vertical movement of the tubes to ensure mixing of the blood, after which it is diluted automatically with diluent. Samples are aspirated in vertically mounted glass pipettes. After 30 minutes, sedimentation is recorded by a light source that scans alongside the pipette and records the difference in absorbance between the plasma and RBC layer.

Measurement of sedimentation using the Ves-Matic Cube 200 system starts with automatic homogenization of selected EDTA-anticoagulated blood samples, followed by loading onto a test tube holder chain. Reading of the initial height is performed by an opto-electronic light source that scans directly through the tube and bar-coding labels present. After 20 minutes, the tube passes the light-emitting diode-based optical system again, and the shift in optical density (represented by the optical density change from plasma layer to RBC layer) is recorded. Temperature correction is applied, and the sedimentation rate is automatically converted to conventional Westergren rates.

The conventional manual Westergren method was applied by diluting 4 volumes of mixed blood with 1 volume
of sodium citrate, according to the ICSH protocol. EDTA-anticoagulated, citrate-diluted blood was aspirated in open-ended, Westergren-type glass pipettes of 300-mm length mounted vertically in a rack. Sedimentation was evaluated visually after 60 minutes.

Statistics

Passing-Bablok regression analysis was performed. Bias and 95% limits of agreement assessment was performed using Bland-Altman analysis. Precision was studied by 10 replicate measurements of samples with low (<20 mm/h), intermediate (20-80 mm/h), and high (>80 mm/h) levels of ESR, and means, SDs, and coefficients of variation were calculated. The Pearson parametric test was used to evaluate correlation (r, correlation coefficient). A Student t test was used for the comparison of groups; P values of less than .05 were considered statistically significant. All data were analyzed by using MS Excel 2003 software (Microsoft, Redmond, WA), Analyse-It version 2.09 (Analyse-It Software, Leeds, England), and SPSS 17 (SPSS, Chicago, IL).

Results

Method Comparison

In total, we measured the ESR in blood samples of 244 patients of 2 hospital centers and general practitioners surrounding these hospitals. The measurement of the ESR in 101 samples (Hoofddorp) resulted in a median ESR using the SEDIsystem of 27 mm/h (95% confidence interval [CI], 2-104 mm/h) and a median ESR of 15 mm/h (95% CI, 5-118 mm/h) using the Ves-Matic Cube 200 system. Passing-Bablok regression analysis between methods resulted in a regression equation of \( y = 1.10x \) (95% CI, 1.00 to 1.18) – 2.87 (95% CI, –5.39 to –2.00 mm/h) \( \text{Figure 1A} \).

For 143 results obtained with the StaRRsed method (Eindhoven), the median ESR was 35 mm/h (95% CI, 5-118 mm/h), and for the corresponding Ves-Matic Cube 200, a median ESR of 22 mm/h (95% CI, 2-82 mm/h) was found. Results of Passing-Bablok regression analysis showed a regression equation of \( y = 0.87x \) (95% CI, 0.78 to 0.96) – 1.30 (95% CI, –2.43 to 0.11 mm/h) \( \text{Figure 1B} \).

Corresponding portioned Bland-Altman analyses showed a significant negative bias, indicating that ESR values measured with the Ves-Matic Cube 200 yield on average 32% lower results than ESR values obtained with the SEDIsystem or StaRRsed methods \( \text{Figure 1C} \) and \( \text{Figure 1D} \). More detailed bias analysis showed that, particularly at low ESR levels (<25 mm/h), the Ves-Matic Cube 200 had a notable negative bias when compared with the SEDIsystem and the StaRRsed. At higher ESR levels, the agreement between methods showed a more random variation.

Comparison With the Westergren Reference Method

To evaluate the accuracy of the obtained ESR results, we additionally performed conventional Westergren analysis according to the ICSH protocol. With Passing-Bablok regression analysis, the SEDIsystem (n = 92) yielded a slope of 0.91 (95% CI, 0.87-0.96) and an intercept of 0.77 (95% CI, 0.09-2.72) compared with the Westergren method. There was a strong correlation between SEDIsystem and Westergren measurements (\( r = 0.96; 95\% \text{ CI}, 0.94-0.97) and no evidence of systemic bias \( \text{Figure 2A} \) and \( \text{Figure 2B} \). StaRRsed sedimentation (n = 50) also showed a strong correlation with the Westergren reference method (\( r = 0.96; 95\% \text{ CI}, 0.93-0.98) although with a steeper slope of 1.22 (95% CI, 1.11-1.33) and an intercept of 3.14 (95% CI, 1.17-6.61). Hence, this resulted in a mean bias of 10.8 (95% limits of agreement between –9.3 and 30.9) \( \text{Figure 2C} \) and \( \text{Figure 2D} \).

Finally, the Ves-Matic Cube 200 method (n = 119) yielded a slope of 0.99 (95% CI, 0.90-1.08) with an intercept of –2.32 (95% CI, –5.44 to –0.81) in Passing-Bablok regression analysis compared with the reference method. However, the Pearson correlation coefficient between the Ves-Matic Cube 200 and the Westergren reference method was only \( r = 0.83 \) (95% CI, 0.76-0.88). This poorer correlation is reflected in a negative bias (–5.7) with considerable 95% limits of agreement (–50.8 to 39.4) \( \text{Figure 2E} \) and \( \text{Figure 2F} \).

This poor correlation between Westergren and the Ves-Matic Cube 200 could lead to different clinical interpretation of results. The current ESR reference range for the Westergren-based methods is 1 to 20 mm/h. Of the 77 samples with an ESR of more than 20 mm/h in the Westergren method, 52 had ESR values obtained with the Ves-Matic Cube 200 that deviated more than 20% from the reference method. More important, in this population, 14 samples (18%) would have been classified as being normal when using the Ves-Matic Cube 200, whereas by using the reference method, these samples would have been classified as being above the reference value. Not once did we find a Ves-Matic Cube 200 result of more than 20 mm/h when the Westergren gave a result of less than 20 mm/h.

Precision and Differential Influence of Variables

These results prompted us to investigate more carefully the analytic and blood sample variables underlying the observed deviations between the Ves-Matic Cube 200 and the Westergren reference method. First, we evaluated whether the aberrations were caused by method imprecision. Precision profiling yielded comparable results at low, intermediate, and high ESR levels between the Ves-Matic Cube 200 and a Westergren-based citrate diluted method \( \text{Table II} \). Thus, observed inaccuracy in the ESR values is most likely not caused by reduced precision of the Ves-Matic Cube 200 method.
To identify variables that could differentially influence the results of the Ves-Matic Cube 200 method other than the Westergren reference method, additional parameters (if available) were retrieved retrospectively from the laboratory information system. Hematocrit level, C-reactive protein (CRP) level, and leukocyte count were retrieved (n = 107 recorded values). A Student t test was performed to determine to what extent these variables were different between groups with and without observed differences in sedimentation results compared with the Westergren reference method [Table 2].

For CRP level and leukocyte count, no significant difference was found between groups of results that deviated more than 20% (lower or higher) from the reference method. However, there was a significant difference in hematocrit level between groups of Ves-Matic Cube 200 results that showed a deviation of more than 20% compared with the Westergren method. Hematocrit levels were significantly lower (P = .001) in the group with Ves-Matic Cube 200 results that were more than 20% higher than Westergren ESR results. Inversely, hematocrit was higher (P < .001).
Method comparison of erythrocyte sedimentation with the Westergren reference method: Westergren vs SEDIsystem (A and B), StaRRsed (C and D), and Ves-Matic Cube 200 (E and F). The gray lines represent the \( y = x \) line (A, C, and E) or the zero bias line (B, D, and F). The bold black line represents the regression line (A, C, and E) or the mean bias (B, D, and F).

A and B. Westergren vs SEDIsystem (n = 92). A, Passing-Bablok regression line, \( y = 0.91x \) (95% confidence interval [CI], 0.87 to 0.96) + 0.77 (95% CI, 0.09 to 2.72 mm/h); Pearson correlation coefficient, \( r = 0.96 \) (95% CI, 0.94-0.97). B, Mean bias, –1.0 (95% CI, –3.3 to 1.2); dashed lines denote 95% limits of agreement (–22.3 to 20.3).

C and D. Westergren vs StaRRsed (n = 50). C, Passing-Bablok regression line, \( y = 1.22x \) (95% CI, 1.11 to 1.33) + 3.14 (95% CI, 1.17 to 6.61 mm/h); Pearson correlation coefficient, \( r = 0.96 \) (95% CI, 0.93-0.98). D, Mean bias, 10.8 (95% CI, 7.9 to 13.7); dashed lines denote 95% limits of agreement (–9.3 to 30.9).

E and F. Westergren vs Ves-Matic Cube 200 (n = 119). E, Passing-Bablok regression line, \( y = 0.99x \) (95% CI, 0.90 to 1.08) – 2.32 (95% CI, –5.44 to –0.81 mm/h); Pearson correlation coefficient, \( r = 0.83 \) (95% CI, 0.76-0.88). F, Mean bias, –5.7 (95% CI, –9.9 to –1.6); dashed lines denote 95% limits of agreement (–50.8 to 39.4).
in the group of samples in which the Ves-Matic Cube 200 method generated ESRs that were more than 20% lower than the Westergren reference method.

Discussion

The ESR is a widely used laboratory test for assessing acute phase inflammation. 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. Although for the management of patients with specific diseases ESR is not always the laboratory test of choice, for general screening purposes, it seems to be equally as useful and reliable as CRP.

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 Westergren-based semiautomated methods has substantially improved the application of ESR measurement. In line with the Westergren reference method, these automated methods dilute whole blood with citrate, measure sedimentation of erythrocytes in dedicated tubes, and, subsequently, recalculate to conventional Westergren units. These automated methods generate fast and reliable ESR measurements and show good correlation with the conventional Westergren 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 Ves-Matic Cube 200 is an example of such a modern automated ESR method that uses standard capped EDTA blood sample tubes for direct measurement of erythrocyte sedimentation.

Herein we report considerable deviations in ESR results between the Ves-Matic Cube 200 and both Westergren-based systems in a group of 244 patients. Most striking was a prominent negative bias present at low ESR levels (<25 mm/h) that eventually resulted in an overall average bias of −32% compared with the Westergren-based methods. Additional conventional Westergren analysis showed a poorer correlation (r = 0.83) for the Ves-Matic Cube 200 method compared with both Westergren-based methods (r = 0.96). This deviation was particularly visible at low ESR levels within the normal range, indicating that in our hands, the Ves-Matic Cube 200 method could not discriminate between ESR levels within the reference range. More important, at higher ESR levels, the Ves-Matic Cube 200 classified 14 samples (18%) as normal (<20 mm/h), whereas the SEDIsystem or StaRrsed method gave results that were above the reference value. This could potentially lead to different clinical interpretation and treatment. It should be noted that a previous report by Perovic et al did not show this negative bias, so whether this observation is exemplary or a general characteristic of the Ves-Matic Cube 200 remains to be investigated. But, also at higher sedimentation rates, the Ves-Matic Cube 200 (as opposed to the Westergren-based methods) showed considerable deviation from the Westergren reference method, which could, in fact, result in relevant overestimation or underestimation of actual ESR levels.

The diminished discrimination between ESR levels within the normal range is perhaps caused by the fact that samples in the Ves-Matic Cube 200 are measured nondiluted
in a standard EDTA tube with a wider diameter compared with the classic Westergren method. This may, in fact, differentially influence the actual packing, aggregation, and sedimentation of erythrocytes, and discrepancies within these processes may be more prominently visible within the normal range. Aggregation of erythrocytes is mainly determined by electrostatic forces. Erythrocytes are negatively charged and will repel one another, thereby decreasing RBC aggregation. The presence of positively charged plasma proteins will neutralize the negative surface charge of erythrocytes and, hence, increase RBC aggregation. With Westergren and Westergren-based methods, the concentration of plasma proteins, such as fibrinogen and gammaglobulin, that cause RBCs to aggregate will be reduced by dilution, leading to reduced RBC sedimentation. The RBCs are also diluted, however, which tends to accelerate sedimentation. It is conceivable that the exact outcome of this balance in methods that use nondiluted samples is difficult to predict. Alternatively, the measurement of sedimentation rates in undiluted blood in a large-bore tube with different layers of bar-coding labels may require an optical system with a resolution that is able to discriminate within the normal range of sedimentation.

The ESR values determined with the manual Westergren reference method showed good correlation with the ESR results generated by the SEDIsystem and StaRRsed methods. The Ves-Matic Cube 200 method showed less correlation with the reference method, reflected by a Pearson correlation of \( r = 0.83 \). Moreover, we found a considerable random bias, especially with ESR values above the reference range, as reflected by the 95% limits of agreement (−50.8 to 39.4 mm/h).

These deviations are not attributable to a greater imprecision because the precision of the Ves-Matic Cube 200 at all ESR levels is comparable to that of a Westergren-based method. This is in agreement with a report that showed an imprecision of the Ves-Matic Cube 200 that was comparable to that of the Westergren reference method. It is interesting that retrospective analysis of all samples with ESR values of more than 20 mm/h revealed a significant difference in the hematocrit level between samples measured with the Ves-Matic Cube 200 that deviated more than 20% from the Westergren reference method. Samples with deviations more than 20% higher using the Ves-Matic Cube 200 method compared with the Westergren reference method had significantly lower hematocrit values. In contrast, hematocrit values were significantly higher in samples that showed ESR values more than 20% lower compared with the Westergren method. Hence, we conclude that the random bias in the Ves-Matic Cube 200 method may be at least partially influenced by the hematocrit level.

It is known that the original Westergren method has a tendency to overestimate ESR values in low hematocrit samples, and this effect can theoretically be reduced by applying the correction of Fabry. Our analysis showed that the Ves-Matic Cube 200 has a tendency to overestimate ESR in low hematocrit samples but moreover underestimates the ESR in samples with normal hematocrit levels. Possibly the algorithm used in the Ves-Matic Cube 200 to correlate results with the Westergren method is based on a fixed hematocrit value or range.

The observed relationship between hematocrit and ESR results in the Ves-Matic Cube 200 may yield nonspecific, high results for hospitalized patients, in whom lower hemoglobin levels are observed more frequently. In contrast, the sensitivity of the ESR value for general screening purposes will be reduced because measurement in patients with normal hematocrit values will generate, on average, lower ESR results. The addition of actual hematocrit measurement in the Ves-Matic Cube 200 and adaptation of the algorithm according to this value might result in ESR measurements that show better correlation with the ICSH Westergren reference method.

This study showed that the Ves-Matic Cube 200 and Westergren-based citrated methods cannot be used interchangeably and that the unsatisfactory correlation cannot be attributed to inaccuracy of the Ves-Matic Cube 200 method. We therefore hypothesize that the observed deviations between the Ves-Matic Cube 200 and the Westergren-based citrated methods are perhaps the result of intrinsic differences between these methods. The process of erythrocyte sedimentation is a physical phenomenon that is influenced by many parameters, including analytic variables (eg, dilution with citrate or test tube) and sample variables (eg, hematocrit level or the presence of monoclonal proteins). It is plausible that the exact outcome and influence of all of these individual variables is difficult (if not impossible) to predict for each individual sample analyzed, thereby severely complicating general algorithmic recalculation to original Westergren ESR results.

We conclude that data resulting from an EDTA-based, directly measured method (such as the Ves-Matic Cube 200 or Test 1) may, in fact, reflect something other than an ESR measured by a diluted Westergren-based method. This hypothesis is in accordance with a comment on a recent study that showed a similar poor correlation between the EDTA-based Test 1 method and the manual ICSH reference method. We strongly agree that one should be reticent about the application of EDTA-based ESR methods before further prospective studies have investigated the informational qualities these methods may hold. Moreover, one should realize that physicians have long made clinical decisions based on an ESR value generated by citrate-diluted, Westergen-based methods. Sudden adoption of an alternative ESR method with a different correlation with the original Westergren method that is differentially influenced by (still) poorly defined variables is, therefore, undesirable.
We have measured the ESR in blood samples of 244 randomly selected hospitalized and general practice patients with a broad range of sedimentation rates. The SEDIsystem and StaRRsed Westergren-based methods showed good correlation with the Westergren reference method and an acceptable bias over the entire range of ESR values. The Ves-Matic Cube 200 method showed poorer correlation with the reference method, which was mainly caused by a considerable negative bias at low ESR values and a more random bias at higher ESR levels. Retrospective analysis showed that Ves-Matic Cube 200 ESR readings at higher levels are at least partially influenced by the hematocrit value of the samples, resulting in a more random bias. The findings of the present study show that the Ves-Matic Cube 200 and Westergren-based methods cannot be used interchangeably. Hence, we conclude that the ESR results of the Ves-Matic Cube 200 are too deviant from the Westergren-based diluted methods to be clinically acceptable.

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