Evaluation of Ves-Matic Cube 200 For Erythrocyte Sedimentation Rate Determination

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Background: International Council for Standardization in Haematology suggested Westergren method as the reference method to analyze erythrocyte sedimentation rate (ESR). However, in recent years closed automated systems that measure ESR directly from a capped EDTA blood sample tube have been developed. We evaluated the analytic performance of one of these new methods, the Ves-Matic Cube 200. **Methods:** K₂EDTA and citrated blood samples were taken from 101 randomly selected outpatients in Ankara Numune Education and Research Hospital. The ESR results using Ves-Matic Cube 200 and Westergren as reference method from 101 patients were compared and interference studies were performed. **Results:** We found

the mean difference between the two methods as 0.19 ± 15.85 mm/hr (–3.317 to 2.940 mm/hr, 95% confidence interval). Regression analysis yielded the equation “ $y = -2.59 + 1.15x$ ” between the two methods ($r = 0.82$). **Conclusions:** Ves-Matic Cube 200 should be monitored carefully for good quality control. Temperature correction should be applied to study control material as recommended by the manufacturer. Ves-Matic Cube 200 device should be monitored carefully, performance studies should be performed, and the results should be checked in order to eliminate the random errors during the routine studies. J. Clin. Lab. Anal. 27:367–372, 2013.

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Key words: ESR; Ves-Matic Cube 200; Westergren; comparison; evaluation

INTRODUCTION

The erythrocyte sedimentation rate (ESR) is a nonspecific test and is not a primary diagnostic tool of any particular disease. However, the measurement of ESR can be used for the follow-up of infectious diseases, inflammatory diseases, malignancies, and autoimmune diseases. ESR is helpful in diagnosing two specific inflammatory diseases such as temporal arteritis and polymyalgia rheumatica. It has a high sensitivity but low specificity. Interpretation of the results should always be along with the patient's clinical history, examination findings, and results of other tests performed for the patient (1–3). Plasma proteins, especially fibrinogen, adhere to the red cell membranes and neutralize the surface negative charges, promoting cell adherence and rouleaux formation. Rouleaux are stacks of many RBCs that become heavier and sediment faster. Increased rouleaux formation contributes to high ESR.

International Council for Standardization in Haematology (ICSH) suggested Westergren method as the reference method to analyze ESR (3). According to West-

ergren method, ESR is measured after the tubes stand vertically for one hour. The Westergren method is not practical for routine use because it is time-consuming and needs large volumes of blood. Therefore various methods have been developed based on the Westergren method to overcome the problems in practice associated with this method. These methods determine the ESR using citrate-anticoagulated tubes such as the StaRRsed (InterRrliner, Mechatronics, Zwaag, the Netherlands) and the SEDIsystem (Becton Dickinson, Leiden, the Netherlands). In addition, some automated systems measuring the ESR directly from a capped EDTA blood sample tube have been developed and are becoming

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increasingly common in our country recently (4). One of these systems is the Ves-Matic Cube 200 (Diesse Diagnostica Senese, Siena, Italy), which is designed to give ESR results from EDTA-anticoagulated blood in 20 min (1). There is no need to draw and transfer the blood, because the instrument executes the analysis of the samples directly from test tubes.

In this study, we aimed to evaluate the analytic performance of the Ves-Matic Cube 200 and compare it with Westergren reference method.

MATERIALS AND METHODS

Patients and Blood Samples

To compare the Ves-Matic Cube 200 and Westergren original method, K₂EDTA (Becton Dickinson System, Europe, Meylan Cedex-France) and citrated blood samples (Becton Dickinson System, Europe, Meylan Cedex-France) were taken from 101 randomly selected outpatients in Ankara Numune Education and Research Hospital and analyzed within four hours of venipuncture, according to the ICSH recommendations. Sedimentation rate of the patients were measured with the Ves-Matic Cube 200 using EDTA-anticoagulated tubes and with the conventional Westergren method using citrated-anticoagulated tubes. Samples with low, medium, and high sedimentation values were used for within-run imprecision. All procedures were approved by our institution's responsible committee in accordance with the Helsinki Declaration of 1975.

ESR Measurement With Ves-Matic Cube 200

Measurement of sedimentation rate in the Ves-Matic Cube 200 was applied by homogenizing the EDTA-anticoagulated whole blood samples and then loading onto a test tube holder chain. Samples were transferred from racks to the test tube holder chain, mixed and transported to the reader point 1. The speed of the chain movement was 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 were measured by an innovative optical system using white light, high power LED (Light Emitting Diode), and analogical photo-sensor. Results were temperature corrected to a temperature of 18°C according to Manley's monogram. Results were obtained using mathematic algorithm for exploration of the results to Westergren values. The first result is obtained after 20 min and the following ones every 18 sec.

Manual Measurement

The reference Westergren method was performed by using citrate tubes, according to the ICSH (5). Four volumes

whole blood was diluted with one volume sodium citrate. The citrate-diluted blood was aspirated in Westergren-type pipettes. Then the filled pipettes were placed vertically in a rack and sedimentation rate was assessed visually after 60 min.

Sample Stability Studies

The ESR of 20 randomly selected patients' samples was measured by Ves-Matic Cube 200 method at fresh, 4, 6, 8, and 24 hr after collection. Ten samples were stored at room temperature and the others were stored at +4°C between analysis.

Interference Studies

The ESR of 10 randomly selected patients samples was measured by the Ves-Matic Cube 200 method. Effect of lipemia and hemolysis was investigated with the addition of total parenteral nutrition (TPN) for lipemia interference studies and hemolyzate for hemolysis interference studies. The dilution rates of TPN and serum were 1:100. To an aliquot of the same serum we added equal proportion of serum physiologic to simulate the dilution from the added lipids. We diluted the serum by adding hemolyzate in 1:10 proportion. To exclude dilution effect, we added the same amount of serum and serum physiologic. The device makes its measurements from the outside of the tube, therefore a possible effect of a contamination to the surface of the tube was also investigated. Treated samples with TPN, hemolyzate, or contamination were entitled as "Spiked" and untreated samples were identified as "Neat." Parameters such as hematocrit, red cell count, and C-Reactive Protein (CRP) that could affect ESR results of the Ves-Matic Cube 200 method were recorded.

Statistical Analysis

Precision studies were performed with 20 replicate measurements of samples with low (<20 mm/hr), medium (20–80 mm/hr), and high (>80 mm/hr) levels of ESR. Means, standard deviations, and coefficients of variation were calculated. A paired Student's *t*-test, Mann-Whitney U test, and Wilcoxon *T*-test was used to compare the means of groups. Values of *P* < 0.05 were accepted as statistically significant. Linear regression analysis was performed according to Passing-Bablok. The bias and limits of agreement were performed using Bland-Altman analysis. The nonparametric test of Spearman's rank correlation was used to evaluate correlation (*r*, correlation coefficient). The calculations were performed by using MedCalc statistical software (ver. 12.3.0.0.; Belgium) and SPSS 13.0 for Windows software (Chicago, IL).

TABLE 1. Evaluation of Within-Run Imprecision at Low, Medium, and High ESR Levels Using Ves-Matic Cube 200

	<i>n</i>	ESR (mm/hr)	Range (mm/hr)	CV (%)
Low (<20 mm/hr)	20	3.27 ± 0.46	3–4	14.06
Medium (20–80 mm/hr)	20	46.05 ± 6.9	33–57	14.99
High (>80 mm/hr)	20	93.1 ± 5.3	85–106	5.69

ESR values are expressed as the mean ± SD, imprecision is expressed as the within-run coefficient of variation (CV).

RESULTS

Evaluation of Within-Run Imprecision

The results of the precision studies are presented in Table 1. Within-run imprecision was 14.01% for the 3 mm/hr, 14.99% for the 46 mm/hr, 5.69% for the 93 mm/hr.

Evaluation of Stability

Results of the stability studies are shown in Table 2. When the specimens are stored at room temperature, ESR results at 24 hr after collection decreased significantly. ESR of refrigerated samples did not change significantly.

Evaluation of Interference

The interference study results are shown in Figures 1–3. The presence of lipids and hemolysis appears to cause a falsely low ESR measurement in Ves-Matic Cube 200 method ($P = 0.005$, $P = 0.008$, respectively; Table 3). Contamination has no effect on the ESR results ($P = 0.281$).

There was a significant positive correlation between CRP levels and Ves-Matic Cube 200 results ($r = 0.323$, $P = 0.002$), but hematocrit level and red cell blood count negative correlated with Ves-Matic Cube 200 results ($r =$

Fig. 1. ESR results of contaminated sample collection tube.

-0.314 , $P = 0.002$; $r = -0.252$, $P = 0.015$, respectively) in all 101 patients.

Method Comparison Study

We evaluated the accuracy of the obtained ESR results using Ves-Matic Cube 200 according to ICSH protocol. ESR was measured in 101 blood samples by the Ves-Matic Cube 200 and Westergren methods. The mean ± SD ESR was 32.36 ± 24.96 mm/hr (95% CI for the mean was 27.43–37.28 mm/hr) for Ves-Matic Cube 200 and was 32.17 ± 27.20 mm/hr (95% CI for the mean was 26.80–37.54 mm/hr) for the Westergren method. We found the mean difference between two methods as 0.19 mm/hr (95% CI for the mean was -3.32 to 2.94 mm/hr; $P = 0.905$). The obtained Spearman's rank correlation coefficient was 0.82 ($P = 0.000$).

TABLE 2. The Results of Evaluation of Stability

	Fresh (<i>n</i> = 10)	4 (<i>n</i> = 10)	6 (<i>n</i> = 10)	8 (<i>n</i> = 10)	24 (<i>n</i> = 10)
Samples stored at RT					
Mean ± SD (mm/hr)	33.10 ± 29.36	34.60 ± 26.70	30.20 ± 26.25	26.60 ± 25.11	5.00 ± 2.78
Mean of differences (mm/hr)		−1.50	2.90	6.50	28.10
95% CI (mm/hr)		−5.20 to 2.20	−2.50 to 8.30	−0.12 to 13.12	6.43 to 49.77
<i>P</i>		0.383	0.255	0.053	0.017*
Samples stored at 4°C					
Mean ± SD (mm/hr)	38.00 ± 34.72	38.40 ± 37.95	37.30 ± 34.70	34.30 ± 32.75	37.50 ± 36.73
Mean of differences (mm/hr)		−0.40	0.70	3.70	0.50
95% CI (mm/hr)		−5.39 to 4.59	−1.38 to 2.78	0.98 to 6.42	−5.17 to 6.17
<i>P</i>		0.860	0.466	0.052	0.846

Measurement of ESR was performed in Ves-Matic Cube 200 both at fresh, at 4 hr, at 6 hr, at 8 hr, and 24 hr after blood samples were drawn and after storage for 24 hr either at 4°C or room temperature (RT). ESR values are expressed as the mean ± SD.

*Significantly different ($P < 0.05$) from the fresh ESR result for the result of at 24 hr.

Fig. 2. Interference studies with TPN.

Passing–Bablok regression analysis yielded the equation “ $y = -2.59 + 1.15x$ ” between the two methods (Fig. 4). The Ves-Matic Cube 200 method ($n = 101$) yielded a slope of 1.16 (95% CI, 1.00–1.35) with an intercept of -2.59 (95% CI, -5.75 to 0.00).

The agreement between results obtained by different methods is demonstrated in different plots according to Bland–Altman. There was no evidence of systemic bias, equal to -0.7 mm/hr (limits of agreement, -32.6 to 31.2 mm/hr) (Fig. 5).

DISCUSSION

ESR is the most widely used test for monitoring infection, inflammatory diseases, trauma, and certain types of

cancer. Although sensitivity of ESR is high, specificity of the test is low (6). Conventional Westergren method mentioned is the reference method for measurement of ESR and for validation of new ESR methods (5).

There are several important disadvantages to analyze sedimentation rate by Westergren method. First, room temperature has an effect on the results and is open to user errors. Second, longer time is required in order to receive the results of patients, such as 30 min, one hour, or two hours. Third, citrated blood sample should be used for measurement of sedimentation rate by Westergren method, which means that a separate sample from the patient for ESR analysis needs to be collected. Finally, it is necessary to perform the measurement within four hours after taking the blood sample.

Fig. 3. Interference studies with hemolyzat.

TABLE 3. The Results of Evaluation of Interference

	Median (Min–Max) (mm/hr) (<i>n</i> = 10)	<i>P</i>
NeatTPN	35 (11–98)	0.005
SpikedTPN	17 (8–58)	
NeatSF	31 (12–101)	
SpikedSF	27 (6–69)	0.025
NeatHemolyzat	33 (14–84)	
SpikedHemolyzat	21 (13–57)	
NeatSF	28 (12–56)	0.008
SpikedSF	24.50 (9–59)

Fig. 4. Comparison of two methods for ESR measurement: Ves Matic Cube 200 and Westergren method.

There are requirements for closed and automated systems in clinical laboratories with regard to safety of laboratory personnel, ease of operation, and a decreased turn-over time. Hence, new technologies have been developed to analyze sedimentation rate, recently (2, 7). In the ESR systems using EDTA, ESR and other hematological parameters can be analyzed with the same EDTA-anticoagulated tube. In this way, cost of health-care can be decreased and less blood is collected from the patients.

In this study, we used Ves-Matic Cube 200 as an example of automated ESR methods using EDTA. We evaluated analytic performance of the Ves-Matic Cube 200 comparing with the Westergren method.

According to literature review, there are no published guidelines for reproducibility in ESR testing. In precision study, it is mentioned that CV% for abnormal control is in the range of 3–7% and for normal control it is reported to be in the range of 15–39% with Diesse Mini-Ves (8). We did not use control material for precision study. The within-run imprecision was 14.01% for the 3 mm/hr, 14.99% for the 46 mm/hr, and 5.69% for the 93 mm/hr. Previously, Perovic et al. (9) had evaluated the Ves-Matic

Fig. 5. Bland–Altman plot of the difference between ESR values obtained with Westergren method and Ves Matic cube 200 against the mean of ESR values in the 101 patients.

Cube 200 system and reported within-run imprecision coefficients for low, medium, and high serum levels (9.19%, 13.88%, 5.66%, respectively), which were comparable with our findings.

Cerutti et al. (10) showed that systematic bias was 1.2 and limits of agreement was –17.4 to 19.9 for the Ves Matic Cube 80 instrument. We found systemic bias as –0.7 and limits of agreement –32.6 to 31.2 in Bland–Altman analysis compared with Westergren reference method. Furthermore, we found a poorer correlation ($r = 0.82$) for the Ves-Matic Cube 200 method compared with Westergren method than Curvers and colleagues did (1).

The stability studies showed that when the specimens are stored at room temperature, ESR results at 24 hr after collection showed differences compared with ESR results of refrigerated samples at day 1 statistically ($P = 0.021$). But Mahlangu et al. (11) found that all ESR analysis using either the HumaSed or the ESR-Auto Plus are not stable beyond four hours.

In the interference analysis, the presence of lipids and hemolysis appears to cause a falsely low ESR measurement in Ves-Matic Cube 200 method as Mahlangu and colleagues found (11). The relationship between ESR values and serum lipid levels is controversial. So, Pawlotsky et al. developed a method as Σ ESR based on undiluted blood collection, manual hematocrit adjustment to a constant value of 0.35, and characterization of the sedimentation curve by summing the values recorded at 20, 30, 40, and 50 min (12). In patients with isolated hyperlipidemia, they found a possible role of the hematocrit value in these contradictory results and showed that Σ ESR could overcome these problems. But contamination has no effect on the ESR results.

Curvers and colleagues showed that Ves-Matic Cube 200 overestimate ESR if the sample has low hematocrit levels (1). We also found negative correlation between ESR results of Ves-Matic Cube 200 and hematocrit levels and red blood count. But CRP levels positively correlated with ESR results of Ves-Matic Cube 200. Ves-Matic Cube 200 should be carefully monitored with quality control assessments if used instead of the reference method. During clinical studies it has been observed that it is affected by hemolysis, contamination on the outer surface of the EDTA tube, and waiting time, which cause a tendency to random errors. Temperature correction should be applied to work control material as recommended by the firm.

CONCLUSION

The results of the study are interpreted as follows: the Ves-Matic Cube 200 device can be used instead of Westergren reference method but should be monitored carefully especially for the conditions causing random errors.

CONFLICT OF INTEREST

None declared.

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