Evaluation of Ves-Matic Cube 200 – an automated system for the measurement of the erythrocyte sedimentation rate

E. PEROVIC, L. BAKOVIC, A. VALCIC

INTRODUCTION

The erythrocyte sedimentation rate (ESR), now more often referred as ‘length of sedimentation reaction of blood’ (LSRB) (Piva et al., 2001), is the most widely used laboratory test of the acute phase inflammatory response (Wolfe & Pincus, 2001). Although the ESR lacks specificity, the test remains helpful in the specific diagnosis of a few conditions, including temporal arteritis, polymyalgia rheumatica and rheumatoid arthritis. It is useful in monitoring these conditions and may predict relapse in patients with Hodgkin’s disease (Haybittle et al., 1985). Recently, it was reported to be of clinical significance in conditions such as stroke and coronary artery disease. In acute coronary syndrome, ESR testing has been described as being of prognostic value and independent predictor of mortality (Wu et al., 2002).

SUMMARY

Ves-Matic Cube 200 is fully automated analyzer that performs erythrocyte sedimentation rate (ESR) measurement using the standard ethylenediaminetetraacetic acid blood sample tube, thus markedly reducing the analytical time and avoiding the need for an extra blood sample. The aim of this study was to assess the automatic Ves-Matic Cube 200 system for the measurement of ESR in comparison with the original International Council for Standardization in Hematology reference method (Westergren). The evaluation comprised accuracy which was established using a 95% confidence interval (CI) for the mean difference between Ves-Matic Cube 200 and Westergren method (mean of difference: 0.47 ± 6.84 mm/h; 95% CI: −0.376 to 1.325 mm/h), within-run imprecision for samples with ESR values of 9, 42 and 95 mm/h (coefficients of variation: 9.19%, 13.88% and 5.66%, respectively) and method comparison (ρ = 0.95; Passing-Bablok regression equation: \[ Y = -0.0435 + 1.0435X \]; bias: −0.5; limits of agreement: −13.9 to 12.9). Stability was estimated after 24 h storage either at 4 °C and room temperature (mean of differences: −1.91 mm/h; 95% CI: −4.852 to 1.037 mm/h and mean of differences: −12.48 mm/h; 95% CI: −16.580 to −8.390 mm/h, respectively). The obtained results suggest that the Ves-Matic Cube 200 automated analyzer is reliable system for the measurement of ESR in clinical laboratories.
The International Council for Standardization in Hematology (ICSH) and The Clinical and Laboratory Standards Institute (formerly The National Committee for Clinical Laboratory Standards – NCCLS) recommend for measurement of ESR recognize reference, standardized and selected methods (ICSH, 1993). The ICSH standardized method has to be directly comparable and traceable with the ICSH reference method. Furthermore, it can be use as an alternative one for verification of the quality control or verification of working (routine) methods. The standardized method is based on the traditional Westergren method, which however uses ethylenediaminetetraacetic acid (EDTA)-anticoagulated samples without dilution. Subsequent ICSH guidelines allow the use of the alternative ESR techniques, if the comparability with the Westergren ESR is achieved. For working (routine) methods, ICSH recommends specifications for selected methods (ICSH, 1993). Several new techniques for measuring ESR have been developed and introduced in clinical laboratories to address the following needs: (i) to guarantee the operators safety using automated and closed systems; (ii) to automate the measurement itself and to optimize the workflow and utilization of human resources; (iii) to create a unique workstation for measuring ESR and performing other hematological tests in a single specimen (Plebani & Piva, 2002).

As suggested by ICSH and NCCLS, this should be achieved using the recommended specimen, undiluted blood with K3-EDTA, which is more reliable than the traditional sodium citrate (ICSH, 1993; NCCLS, 2000). This type of anticoagulated sample has several advantages. In particular, it preserves the red blood cell (RBC) morphology and does not interfere with mechanisms that lead to erythrocyte sedimentation. Furthermore, it increases specimen stability and reduces the risk of pre-analytic mistakes caused by small blood clots or partially coagulated specimens. Therefore, it does not incur problems related to sample dilution with sodium citrate, because the ratio between blood and anticoagulant is of the crucial importance (Plebani, 2003).

The present study aimed to evaluate ESR measurement by this new Ves-Matic Cube 200 analyzer of K3-EDTA blood samples and to compare the results with the original Westergren method. Analytical evaluation was performed by determination of accuracy that represents comparability because of methodological reasons, precision and stability.

### MATERIALS AND METHODS

This prospective study included blood samples from 257 patients (111 males and 146 females, aged 18–88 and 19–82, respectively) that were selected randomly from the entire population of both hospitalized and ambulatory patients. The Ethic Board approved the evaluation study at Zadar General Hospital. Paired blood samples were obtained by venipuncture into K3-EDTA (each containing 0.072 ml 7.5% K3-EDTA per 3.0 ml blood) and sodium citrate vacuum tubes (each containing 0.4 ml 0.129 M sodium citrate per 1.6 ml blood) (Vacutainer, Becton Dickinson, UK). The samples were processed under standardized conditions (collected in the morning after all night fasting) and tested within 4 h of venipuncture. Blood samples were distributed as follows:

- Six samples with normal and pathological ESR values for evaluation of the within-run imprecision: 10 replicate measurement of three samples with ESR values of 9, 42 and 95 mm/h for Ves-Matic Cube 200; 10 replicate measurement of three samples with ESR values of 9, 59 and 90 mm/h for Westergren method.
- Two hundred and fifty-one samples for comparison between Westergren method and Ves-Matic Cube 200.
- One hundred and thirty-three samples (from those 251 used for comparison study) for testing stability between fresh and 24 h stored blood [at 4 °C (n = 65) or at room temperature (n = 68)].

### Description of the Ves-Matic Cube 200 analyzer

Ves-Matic Cube 200 (Diesse Diagnostica Senese SpA, Siena, Italy) is a closed automatic system for determining ESR in K3-EDTA or K2-EDTA tubes that is able to analyze up to 180 blood samples per hour. Venous samples were collected in standard 3 ml, vacuum, purple-top, K3-EDTA tubes and inserted into hematology analyzer rack. The ESR test was performed without aerosol produced by eventual perforations of the caps. The sample loader provides continuous and random loading from racks used by automated hematology analyzers. 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 opto-electronic elements (white light, high power LED and analogical photo-sensor). The measurement was monitored from phase of the rouleaux formation, through the phase of sedimentation, till the phase of red cells packing. Results were temperature corrected to a temperature of 18 °C according to Manley’s nomogram. The ESR was affected by room temperature. Manley (1957) developed a nomogram to correct for variation in room temperature (15–35 °C) by following equation:

\[
\text{corrected ESR in mm in first hour} = (A2 \times (\text{observed reading})^2 + (A1 \times \text{observed reading}) + A0)
\]

Results were processed using mathematic algorithm for extrapolation of the results to Westergren values. The first result is obtained after 20 min and the following ones every 18 s.

**Manual measurement**

The ESR measurement was performed according to ICSH’s specifications but using citrate tubes (Vacutainer, Becton Dickinson, UK) and disposable plastic pipettes (Vacuette, Greiner Bio-One GmbH, Austria) in order to reduce biological hazards. Venous samples were collected in standard 2 ml, vacuum, black-top, sodium citrate-containing tubes. The test tubes were then manually gently mixed back and forth 10 times, and plastic pipette was inserted into the test tubes until column of blood rose to the start stopper. The graduated, filled pipettes were placed vertically into stand and after 1 h, the sedimentation of the RBC’s was recorded through visual determination. All samples were kept at room temperature and run within the ICSH-recommended 4-h period. A very close correlation between the ESR measurement performed with this modified method and with the classic method using glass pipettes (ICSH’s method) as observed previously in this laboratory 15 years ago (Car M, Perovic E and Bakovic L, unpublished data).

**Statistical analysis**

Data are reported as the mean ± standard deviation (SD) and a paired Student’s t-test was used to compare the means. Accuracy was evaluated using a 95% confidence interval (CI) for the mean difference between methods. There is no evidence of systematic bias when 95% CI includes zero. Imprecision test was performed evaluating means, SD and coefficients of variation (CV). Non-parametric test of Spearman was used to evaluate correlation (ρ, correlation factor). Linear regression analysis was performed according to Passing-Bablok. The bias and limits of agreement were performed using Bland–Altman analysis. All statistical calculations were performed using Microsoft Excel 2003 and MedCalc program (ver. 9.0.1.0.; Franck Schoonjans, Belgium). Values of \(P < 0.05\) were considered statistically significant.

**RESULTS**

**Evaluation of within-run imprecision**

The results of evaluation of within-run imprecision are presented in Table 1 and CVs did not differ significantly between the methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>ESR (mm/h)</th>
<th>Range (mm/h)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (9 mm/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ves-Matic Cube 200</td>
<td>10</td>
<td>8.7 ± 0.8</td>
<td>7–10</td>
<td>9.19</td>
</tr>
<tr>
<td>Westergren</td>
<td></td>
<td>8.6 ± 0.8</td>
<td>7–10</td>
<td>9.30</td>
</tr>
<tr>
<td>Medium (42 mm/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ves-Matic Cube 200</td>
<td>10</td>
<td>42.5 ± 5.9</td>
<td>36–50</td>
<td>13.88</td>
</tr>
<tr>
<td>Westergren</td>
<td></td>
<td>58.8 ± 8.4</td>
<td>47–74</td>
<td>14.29</td>
</tr>
<tr>
<td>High (95 mm/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ves-Matic Cube 200</td>
<td>10</td>
<td>95.4 ± 5.4</td>
<td>87–104</td>
<td>5.66</td>
</tr>
<tr>
<td>Westergren</td>
<td></td>
<td>89.8 ± 5.3</td>
<td>81–100</td>
<td>5.90</td>
</tr>
</tbody>
</table>

The degree of imprecision in the measurement of erythrocyte sedimentation rate (ESR) was assessed by analyzing blood samples with normal and high ESR values by both the Ves-Matic Cube 200 and Westergren method. ESR values are expressed as the mean ± SD of 10 determinations, whereas imprecision is expressed as the within-run coefficient of variation (CV).

**Evaluation of stability**

The evaluation of stability showed that there was no statistical difference of ESR when specimens were...
stored at 4 °C ($P = 0.2003$). There was statistically significant difference of ESR when specimens were stored at room temperature ($P < 0.0001$). The results are shown in Table 2.

### Method comparison study

The ESR measurement performed using Ves-Matic Cube 200 and Westergren methods from 251 patients were compared. Mean ESR value measured with Ves-Matic Cube 200 method was 18.90 mm/h; (95% CI for the mean was 16.28–21.52 mm/h) and there was no significant difference from those measured with the Westergren method (mean was 19.38 mm/h; 95% CI for the mean was 16.89–21.86 mm/h). Mean of differences was 0.47 mm/h; 95% CI for the mean was $0.37$ to $1.32$ mm/h; $P = 0.2734$. The obtained Spearman’s rank correlation coefficient was 0.946 ($P < 0.001$) (Figure 1).

Results of linear regression analysis according to Passing-Bablok showed very good correlation between these methods with obtained linear regression equation $Y = -0.0435 + 1.0435$ (Figure 1).

The agreement between results obtained by different methods is demonstrated in different plots according to Bland–Altman. There was no evidence of systemic bias (bias = −0.5) and limits of agreement were −13.0 to 12.9 mm/h (Figure 2).

### DISCUSSION

The erythrocyte sedimentation reaction is a nonspecific phenomenon that might denote the presence or severity of the particular pathological processes. One of the most important applications of ESR is in screening for the presence of more or less occult disease and therefore it is considered a valuable routine procedure (NCCLS, 2000). The ESR maintains an important role in the diagnosis and management of the patients with rheumatoid arthritis, giant cell arteritis, and polymyalgia rheumatica and can also be quite useful in diagnosis of osteomyelitis, some infections and various cancers. On the other hand, ESR was shown to be a good predictor of coronary heart disease mortality and appeared to be a marker of aggressive forms of the this disease (Erikssen et al., 2000). In addition to the information given by fibrinogen regarding the risk of coronary heart disease death, ESR provides other substantial information (Wu et al., 2002).

The ESR is not the measure of an analyte, but the measure of a physical phenomenon depending on a large number of variables (number and size of erythrocytes, plasma density, protein concentration and antibodies). Westergren method and its modified versions measure neither the kinetics nor the rate of erythrocyte sedimentation, but only the final phenomenon described by Fahraeus (1921).

The term ‘LSRB’ is proposed as a general substitute for ESR, even in connection with Westergren’s technique and the ICSH reference method. The internationally recommended systematic description of the distance that erythrocytes settle within a special tube in a fixed time is LSRB (International Union of Pure and Applied Chemistry, International Federation of Clinical Chemistry, 2000). It is still considered, an unanswered question; whether the erythrocyte sedimentation has to be measured as rate (the reaction in period of time) or length (the period of time is

### Table 2. The results of evaluation of stability

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>Mean (mm/h)</th>
<th>Range (mm/h)</th>
<th>CV (%)</th>
<th>Mean of differences (mm/h)</th>
<th>95% CI (mm/h)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>68</td>
<td>15.1</td>
<td>1–92</td>
<td>1.21</td>
<td>−12.4853</td>
<td>−16.5804 to −8.3902</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>24 h stored at RT</td>
<td>68</td>
<td>2.6</td>
<td>1–36</td>
<td>1.73</td>
<td>−1.9077</td>
<td>−4.8525 to 1.0371</td>
<td>0.2003</td>
</tr>
<tr>
<td>Fresh</td>
<td>65</td>
<td>9.6</td>
<td>1–60</td>
<td>1.04</td>
<td>−1.9077</td>
<td>−4.8525 to 1.0371</td>
<td>0.2003</td>
</tr>
<tr>
<td>24 h stored at 4 °C</td>
<td>65</td>
<td>7.7</td>
<td>1–57</td>
<td>1.20</td>
<td>−1.9077</td>
<td>−4.8525 to 1.0371</td>
<td>0.2003</td>
</tr>
</tbody>
</table>

Measurement of erythrocyte sedimentation rate (ESR) were performed in Ves-Matic Cube 200 both within 4 h after blood samples were drawn (fresh) and after storage for 24 h either at 4 °C or room temperature (RT). ESR values are expressed as the mean ± SD of 65–68 blood samples.

© 2009 The Authors
irrelevant). Because different phases of erythrocyte sedimentation depend on time it might be concluded that term LSRB should be reserved for analysis performed with the systems which do not follow Westergren-like technique (Hardeman, 2007).

As the ICSH reference method is performed with EDTA sample in narrow and long tubes, the samples with hematocrit values >0.35 may give poor reproducibility. Therefore, an alternative Westergren method with diluted citrate sample brings all hematocrit values ≤0.35, causing modifications of blood behavior during the phase of sedimentation. The relation between sedimentation in EDTA and citrate is the following: for the interval 15–105 mm/h, sedimentation in citrate = \[ (\text{sedimentation in EDTA} \times 0.86) \] 12. As evinced by this relation, the value of EDTA sedimentation is higher than the citrate one. According to the ICSH and NCCLS
recommendations, the correspondent citrate range for
given EDTA value is wide enough (ICSH, 1977).

The collection of specimens with K$_3$-EDTA
enhances blood cell stability, thus favoring rouleaux
formation, preserving morphologic features, and pre-
cluding nonphysiologic effects on the cells, and conse-
quently being of a great importance for the ESR
reaction (Plebani & Piva, 2002).

As the phenomenon of erythrocyte sedimentation
is transient and confined to fresh blood, presently the
only feasible way to provide a control material is to
specify a method for the production of such material
in the laboratory where it will be used. Because of the
nature of the human erythrocyte sedimentation reac-
tion, reference or control materials of the usual type
are not available for the ESR test (NCCLS, 2000). Ple-
biani _et al._ reported that stabilized specimens of human
or nonhuman origin cannot be considered acceptable
in place of fresh human blood, nor suitable for use in
methods which measure the kinetics of RBC sedimen-
tation (Plebani & Piva, 2002).

The analyses undertaken with the purpose to
determine the within-run imprecision for the auto-
matic Ves-Matic Cube 200 system were performed
according to ICSH recommendations for measurement
of ESR. The imprecision obtained over a range of ESR
values was satisfactory, but it increased at medium
and low rates of sedimentation (from CV of 5.66% for
high value, 13.88% and 9.19% for medium and low
value, respectively). CV values obtained by studied
method did not differ significantly from those
obtained by Westergren method. It was previously
observed that the reduced precision at low rates of
sedimentation does not affect the clinical reliability of
measurements (Plebani _et al._, 1998).

The major limitation of ESR measurement is the
necessity to run the test within 2 h of venipuncture
when blood is stored at room temperature, or within
6 h when stored at 4 °C (ICSH, 1977). In our study,
when the effects of blood storage were studied, the
highest differences were obtained when the measure-
ments were performed after 24-h storage of the sam-
plies at room temperature (mean of differences was
−12.4853 and 95% CI was −16.5804 to −8.3902;
P < 0.0001). It was probably caused by marked eryth-
rocyte swelling (Thomas & Karpic, 1993), or decrease
in the sialic acid content of the erythrocyte membrane
during storage (Lugton, 1989).

The results obtained by Ves-Matic Cube 200 ana-
lyzer provided an accurate value of ESR in most of
the samples when compared with Westergren method
(95% CI −0.3765 to 1.3247 mm/h; _P_ = 0.2734).
Comparability testing represents no systemic bias when
the 95% CI includes zero.

As well, the results obtained by studied method
indicate good correlation (_r_ = 0.946) with Westergren
method measurement. Furthermore, linear regression
analysis according to Passing-Bablok indicated high
concordance between the results obtained by studied
analyzer and those obtained by Westergren method.
Bland–Altman analysis demonstrated no evidence of
systematic bias and obtained limits of agreement are
satisfactory, although there was a slightly dispersion
of the differences above value of 50 mm/h for ESR
measurements. The same dispersion of higher ESR
results was also found by authors who evaluated
EDTA blood samples found when EDTA blood based
instruments which measured ESR by other techniques
(de Jonge _et al._, 2000; Ozdem _et al._, 2006). One of the
plausible reasons could be that measurements take
place at different time intervals and at different phases
of sedimentation reaction. If the descent of the packed
RBCs is plotted against time, it forms a typical sigmoid
curve with three distinct phases. The initial portion of
the curve, the lag phase, reflects red cells rouleaux
formation. The size of the aggregates formed in this
phase is critical for the outcome of sedimentation.
During the second, decantation phase, the aggregated
RBCs fall more rapidly, whereas during the final
phase, cell aggregates pile up on the bottom of the
tube (Koepke, 2002) The sedimentation time for each
phase may vary from patient to patient and the choice
of the interval becomes arbitrary. The Westergren
method measures the final contributions resulting
from all three phases, which represent the fall of ery-
throcytes during 60 min. Altogether, new instruments
which utilize innovative techniques shorten the test-
ing time. The majority of them estimate the final fall
of sedimentation phenomenon, selecting an appropri-
ate time interval to measure red cell sedimentation
(Piva _et al._, 2006).

In conclusion, the Ves-Matic Cube 200 analyzer
offers a fast determination of ESR with acceptable
accuracy and imprecision and good correlation with
reference Westergren method. The use of samples
with EDTA as anticoagulant instead of sodium citrate

© 2009 The Authors

Article in press; Revised version: November 2009

reduces the risk of pre-analytic errors. This procedure allows the use of the same standard EDTA tube for multiple hematological analyses, thus avoiding biological hazard and reducing blood sample volume taken from the patient. In a nutshell, all these findings indicate that Ves-Matic Cube 200 is reliable and suitable system for high workload clinical laboratory.

Acknowledgement

The authors would like to thank to Ivana Valcic and Ante Gambiraza from Hematology Division of Department of Laboratory Diagnostics, Zadar General Hospital, for processing the samples for this study.

References


Hardeman MR (2007) ESR or LSRB, that is the question! Clinical Chemistry and Laboratory Medicine 45, 124.


