Ves-Matic CUBE 200: is modified Westergren method for erythrocyte sedimentation rate a valid alternative to the gold standard?

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ABSTRACT

Ves-Matic CUBE 200 is an automated erythrocyte sedimentation rate (ESR) analyser based on the modified Westergren principle of measurement. In this study, we aimed to assess its analytical performance following the key points addressed by the International Council for Standardization in Haematology and the comparability with the gold standard Westergren method. Comparison of the two methods yielded a correlation coefficient of 0.852, no significant bias and a small constant difference between compared results. Intrarun coefficients of variation (CV) ranged from 2.2% to 22.2%, the higher being for lower ESR values, while inter-run CVs were 19.7% for the normal range and 3.0% for the abnormal range. This study proved the analytical validity of the Ves-Matic CUBE 200 and its high comparability with the Westergren method, showing obvious improvements in the technology applied for automated determination of ESR and a valuable step forward in standardisation of ESR methods.

INTRODUCTION

The erythrocyte sedimentation rate (ESR) is a laboratory test introduced in the early 1900s and still one of the most commonly prescribed and widely used tests in many haematology laboratories. Since its early days, the ESR, more than a speed, is the evaluation of the length of sedimentation of red blood cells (RBC) in a specific pipette during an established period of time.[1 2] The process of erythrocyte sedimentation is described into three characteristic phases that include aggregation of RBCs into rouleau formations, their precipitation and sedimentation and, finally, erythrocyte packaging. Aggregation is the phase that mostly affects the overall sedimentation process, and is enhanced by the presence of negatively charged plasma proteins, mainly acute phase reactants.[3 4] Clearly, a number of other physiological and pathophysiological conditions affect this process, thus making ESR a non-specific marker of inflammation that is still considered helpful when used appropriately in selected clinical conditions,[5 6] specifically in rheumatological diseases where it is incorporated in diagnostic criteria.[7]

The method originally described by Westergren in diluted whole blood by estimating the effects of all three sedimentation phases has been endorsed as the gold standard method by the International Council for Standardization in Haematology (ICSH).[1] This method is simple to perform and relatively cheap, but its original performance is time consuming and requires a relatively large amount of blood.[8] That is why throughout the last two decades a number of new semiautomated and automated ESR methodologies have been proposed that aimed to reduce drawbacks of the Westergren method while keeping its benefits. According to the ICSH working group, methods are classified as 'modified Westergren' if they are based on the original Westergren methodology with minor modifications including shorter assay time or different anticoagulant, or 'alternate methods' if they incorporate completely novel methodological principles.[1 2 9]

This recent development of a large number of new methods has provided advantages such as the use of standard EDTA tube, shorter analysis time and greater safety for laboratory personnel, thus providing new perspectives in ESR testing. The renewed interest on the topic has determined further elaboration on prerequisites prior to introduction of these methods into routine practice and the need for quality procedures to ensure accurate and reliable determinations.[1] Here we report the analytical validation of a modified Westergren method applied on the Ves-Matic CUBE 200 and its comparability with the gold standard Westergren method.

MATERIALS AND METHODS

Setting and study design

The study was performed at the Department of Laboratory Medicine of the University-Hospital of Padova, Italy. Validation of the Ves-Matic CUBE 200 analyser was carried out following the key points of the recommendations published by the ICSH[1] and included determination of intrarun and inter-run precision, assessment of sample stability, haemolysis interference and comparison with the gold standard Westergren method. Intrarun precision was studied by five replicate measurements of 11 patient samples covering the whole ESR analytical range, and reported as mean, SD and coefficient of variation (CV). Inter-run precision was obtained from analysis of commercial control samples (ESR CONTROL CUBE; Diesse Diagnostica Senese, Siena, Italy) on two levels (normal and abnormal) in triplicate for 5 consecutive days, CV (%) was reported. Method comparison with the Westergren method included 448 routine patient samples spanning the whole ESR range. The samples were

further divided into groups of low (<40 mm), middle (40–80 mm) and high ESR values (>80 mm), and statistical analysis was performed accordingly. Non-parametric Spearman's rank coefficient (ρ) was used to evaluate correlation between compared data. Passing-Bablok regression analysis, assisted by bias analysis according to Bland-Altman, was used for statistical analysis of method comparison.

Sample stability was determined on 13 randomly chosen patients who had two EDTA blood tubes drawn for routine laboratory diagnostics. ESR was first measured on the Ves-Matic CUBE 200 analyser in fresh samples within the shortest possible time interval after blood draw, and after 4, 8, 24 and 48 hours' storage, both at room temperature (RT) and 4°C. Refrigerated samples were allowed to return to RT prior to analysis. Paired samples t-test was used for comparison of groups, $p<0.05$ was considered statistically significant.

Haemolysis interference was assessed by analysing 35 patient EDTA samples that were sent for haematological analysis and had a haemolysis index (HI) above 1.0 g/L (ranging from 1.13 to 4.71 g/L) in a paired biochemical sample obtained from the same venipuncture. Samples were analysed on Ves-Matic CUBE 200 and with the manual Westergren method. The HI was determined on Cobas 8000 modular analyser series (Roche Diagnostics, Mannheim, Germany). The samples were grouped according to the HI value in three groups and the median HI value was calculated. To assess the haemolysis effect on ESR determination, the bias for each sample and an average bias for each HI category were calculated according to the following formula:

$$\text{Bias (\%)} = ((\text{Ves-Matic CUBE 200 ESR} - \text{Westergren ESR})/\text{Westergren ESR}) \times 100$$

All blood samples used in the study were anticoagulated with K_2-EDTA (Vacutainer, Becton Dickinson, Plymouth, UK) and selected from routine patients, both hospitalised and ambulatory, who had an initial request for haematology analyses (complete blood count and/or ESR). All samples were leftovers from daily routine, either destined to discarding. The study was conducted in concordance with the principles of the Declaration of Helsinki.

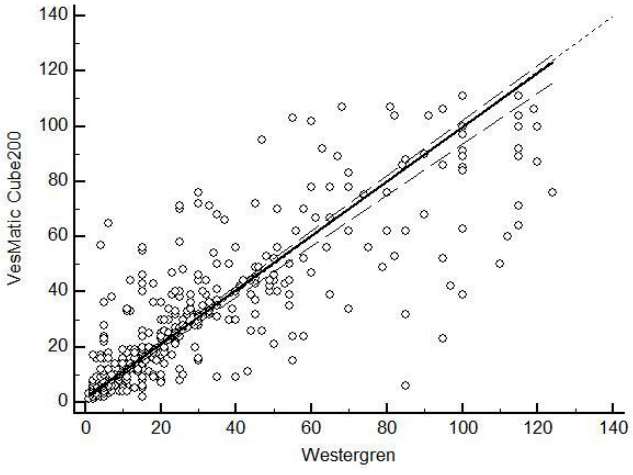

Figure 1 Passing-Bablok analysis for comparison of Ves-Matic CUBE 200 and the Westergren method; y=1.4+0.98x, intercept A 1.4 (95% CI 1.0 to 2.1) and slope B 0.98 (95% CI 0.93 to 1.0).

Table 1 Intrarun precision obtained by analysing 11 patient samples in five replicates

	ESR (mm) (n=5)	SD (±)	CV (%)
S1	4.6	0.5	10.7
S2	10.2	1.3	13.0
S3	15.2	0.8	4.9
S4	23	5.1	22.2
S5	33.2	1.6	4.8
S6	41	1.9	4.6
S7	56.4	1.5	2.7
S8	68.2	4.8	7.0
S9	75.4	2.6	3.4
S10	85.6	2.1	2.4
S11	107.8	2.4	2.2

CV, coefficient of variation; ESR, erythrocyte sedimentation rate.

Description of the Ves-Matic CUBE 200 analyser

Ves-Matic CUBE 200 is an automated ESR analyser that measures ESR from primary EDTA tubes by applying a modified Westergren method. The analyser uses sample racks from automated haematology analysers that are positioned onto the sample loader that allows continuous loading, with a declared analytical capacity of 190 samples per hour that was confirmed in our experience. Samples are transferred to the test tube holder chain and processed accordingly. First, automated mixing of samples is performed for 2 min to ensure complete disaggregation of erythrocytes. Samples are then transported to the first reading point where the height of the sample column is detected and the samples are allowed to settle for 20 min. Using an optical reading system, the analyser determines the difference between the heights of the RBC column before and after sedimentation at the second reading point, with the first result being available within 22 min, and the following every 18 s. Finally, the obtained results are extrapolated to 60 min values to correlate with the Westergren method and temperature correction to 18°C is applied.[10 11]

Westergren method

As described in the ICSH recommendations,[1] samples were diluted with sodium citrate in a proportion of 4 volumes of anticoagulated blood with EDTA and 1 volume of citrate. The samples were thoroughly mixed by complete inversion 10 times, both prior dilution preparation and aspiration into pipettes. The diluted sample was aspirated in disposable glass pipettes with suction piston, with a length of 210 mm±1 mm, an external diameter of 4.45 mm±0.13 mm and a thickness of 1 mm±0.04 mm which have been allocated in a specific support rack to ensure a perfectly vertical position. The racks were positioned in a fume hood at a constant temperature of 18°C–25°C, and protected from light and other potentially influencing external disturbances. The ESR on the graduated scale was read visually after 60±1 min and expressed as millimetres.

RESULTS

The results of intrarun precision using patient samples are presented in table 1.

Analysis of commercial control samples 5 days in triplicate yielded an intrarun CV of 22.1% for the normal range, and 2.8% for the abnormal range. Obtained inter-run CVs were 19.7% and 3.0%, respectively. Grubbs' test did not reveal outliers.

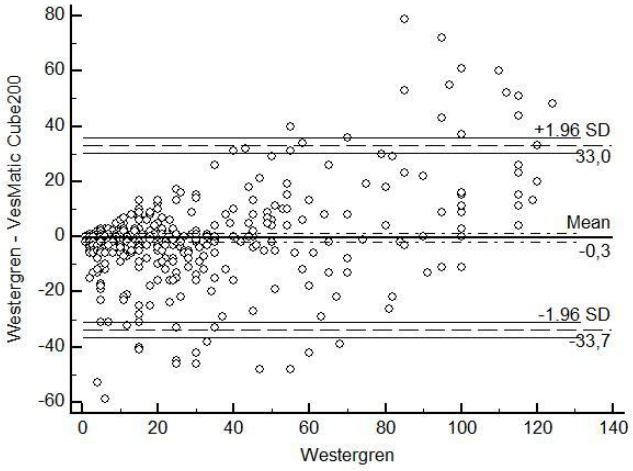

Figure 2 Bland-Altman analysis for comparison of Ves-Matic CUBE 200 and the Westergren method; bias=−0.3 (95% CI −1.9 to 1.2), with value of 33 for the upper limit and −33.7 for the lower limit.

Method comparison

Measurement of ESR in 448 samples resulted in a median of 18 mm (IQR: 6–40) using Ves-Matic CUBE 200 and 19 mm (IQR: 8–42) with the Westergren method. The obtained Spearman's rank correlation coefficient was 0.852 (95% CI 0.824 to 0.875, p<0.001). Figure 1 summarises the results of Passing-Bablok regression analysis, while figure 2 displays the Bland-Altman residual plot distribution of difference around fitted regression line. Comparison of the two methods yielded a non-significant bias and a small constant difference between compared results.

Additionally, results of method comparison of ESR values when evaluated separately per each third of the analytical range, that is, the low (<40 mm), middle (40–80 mm) and high parts (>80 mm), are presented in table 2.

Results of assessment of haemolysis interference and sample stability are presented in tables 3 and 4, respectively.

DISCUSSION

The results of our study show high comparability of the modified Westergren method applied to the Ves-Matic CUBE 200 analyser with the gold standard Westergren method as well as its analytical validity.

An overall non-significant bias was evidenced by Bland-Altman analysis, indicating high concordance of results obtained with the Ves-Matic CUBE 200 in comparison with Westergren and implying the possibility of introducing Ves-Matic CUBE 200 in routine practice as a valid substitute of the reference method. The obtained correlation coefficient was similar to those reported in previously published validation studies[10 11] and shows good correlation with the Westergren method while Passing-Bablok regression analysis yielded a small constant difference between the compared methods. These minor discrepancies can be attributed to different ESR measurement time points, as ESR does not represent a well-defined measurand but rather is a result of a complex physicochemical reaction. However, as already reported in a previous study,[11] the comparability between ESR results was the highest for the low analytical range, while the high analytical range (>80 mm) yielded low coefficient of correlation and a larger dispersion of results, evidenced by a large positive bias with considerable 95% limits of agreement and both constant and proportional difference between obtained results. This observation can be attributed to different measurement time points, a smaller sample size in this range as well as two individual samples identified as outliers with unusually low ESR results (6 mm) obtained with Ves-Matic CUBE 200 compared with 85 mm for both samples measured by the Westergren method. The issue of these two outliers remains unclear and we can speculate that was caused by a random analytical error, either wrong visual reading of the Westergren result or an unidentified cause of erroneous optical reading by Ves-Matic CUBE 200. Although this could surely deserve further investigation, still the vast majority of ESR results fell within the same subgroup, meaning that with both methods patients would be equally classified in most cases. Therefore, observed poorer correlation in this range is a consequence of results variation rather than poor analytical performance, which is expected due to different measurement time points. Similar to previously published studies, the highest intrarun CVs were obtained for low ESR values, while imprecision of intermediate ESR values was much better in our study as compared with others.[10–12] The only exception was a high intrarun CV for the sample with the ESR value of 23 mm (22.2%), but since all other CVs are much lower, this observation can be considered as an isolated case rather than a general characteristic of the Ves-Matic CUBE 200.

Equal to other validation studies that dealt with sample stability,[9 11 12] we demonstrated that there is no statistically

Table 2 Results of method comparison for ESR results between Ves-Matic CUBE 200 and the Westergren method, divided per low, middle and upper ESR range

	Low range (<40 mm)	Middle range (40–80 mm)	Upper range (>80 mm)
n	336	62	50
ρ (95% CI)	0.782 (0.737 to 0.820)	0.512 (0.301 to 0.676)	0.288 (0.011 to 0.524)
Intercept (95% CI)	−0.2 (−1.0 to 0.7)	−92.4 (−152.2 to −55.5)	−176.9 (−436.0 to −78.1)
Slope (95% CI)	1.2 (1.1 to 1.3)	2.7 (2.0 to 3.9)	2.6 (1.6 to 5.2)
Mean bias (95% CI)	−4.3 (−5.4 to −3.1)	1.9 (−3.2 to 7.0)	23.4 (16.1 to 30.7)

Table 3 Results of haemolysis interference (HI) on ESR results

HI range (g/L)*	n	Median (g/L)	Mean bias, % (95% CI)
1.0–1.5	11	1.2	−18 (−44 to 7)
1.5–2.5	12	1.9	−14 (−39 to 12)
2.5–5.0	10	3.2	−41 (−60 to −23)

*HI range was reported as g/L of haemoglobin.
ESR, erythrocyte sedimentation rate; HI, haemolysis index.

Table 4 Results of assessment of sample stability for Ves-Matic CUBE 200

Fresh		Mean (mm) 26.2	Mean difference, mm (95% CI)	P value
Stored at RT (hour)	4	25.8	−0.4 (−8.0 to 7.2)	0.914
	8	19.7	−6.5 (−19.0 to 5.9)	0.275
	24	9.3	−16.9 (−30.9 to −2.9)	0.022
	48	3.2	−23.0 (−39.1 to −7.0)	0.009
Stored at 4°C (hour)	4	25.8	−0.5 (−6.7 to 5.8)	0.874
	8	24.8	−1.4 (−6.9 to 4.1)	0.594
	24	19.2	−7.1 (−16.8 to 2.6)	0.137
	48	19.2	−7.0 (−13.6 to −0.5)	0.038

RT, room temperature.

significant difference of ESR values after 24 hours' storage at 4°C but that results drastically fall when samples are stored at RT. Additionally, our study was the first to assess sample stability after 48 hours' storage, and observed a statistically significant difference, thus such long storage period is not acceptable for ESR determination on Ves-Matic CUBE 200.

Haemolysis in the studied range showed to cause an increasingly negative bias on ESR values with rising haemolysis. Our approach for haemolysis interference assessment included analysis of routine haemolysed samples, without additional haemolysis induction and differs from the one reported in the studies by Sezer *et al*[12] and Boğdaycioğlu *et al*[13] on Ves-Matic CUBE 200 that was based on spiking of native samples with a haemolysate solution. However, the latter studies also showed significant decrease in ESR levels for haemolysed samples.

As demonstrated in our study, the Ves-Matic CUBE 200 is a fast, reliable and versatile system for ESR analysis. Its many advantages compared with the manual Westergren method include measurement of ESR from the same sample used for other haematology analyses, that is, the EDTA undiluted sample, in that way enhancing patient safety, complete automation of ESR measurement that excludes the possibility of operators' manual errors or subjectivity in reading of ESR results and the possibility of connectivity with available haematology analysers. Continuous loading of samples using the same sample racks from haematology analysers makes it suitable for high-throughput laboratories and can contribute to an improved workflow. Moreover, since Ves-Matic CUBE 200 optically measures settling of RBCs without sample consumption, no blood is withdrawn nor liquid waste is produced, which makes this analyser attractive in terms of saving limited sample amount that can be used for other laboratory analyses as well as decreasing biohazard production.

CONCLUSION

Our study shows satisfactory concordance of ESR results from Ves-Matic CUBE 200 with the Westergren method arising from obvious technological improvements in novel ESR technologies which is surely a step forward in the process of harmonisation of ESR measurement. Ves-Matic CUBE 200 as a modified Westergren method provides accurate determination of ESR in a shorter period of time compared with the performance of the Westergren method. Therefore, their interchangeable use can be applied but clearly only after method validation following ICSH recommendations was performed. In that way, traceability of ESR results obtained with different available methods is guaranteed, thus not compromising patient care.

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Contributors IL performed the study, analysed and interpreted the data, and wrote the manuscript. EP designed and performed the study, analysed and interpreted the data, and cowrote the manuscript. FS and GM performed the study and analysed the data. FT and MP designed and performed the study. MP designed the study and critically reviewed the manuscript. All authors drafted the article and approved its final version for submission.

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