

ORIGINAL ARTICLE

Performance Evaluation of the Alifax Test1 2.0 for Erythrocyte Sedimentation Rate Testing

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ABSTRACT

Background: The erythrocyte sedimentation rate (ESR) is widely measured using modified or alternative methods. The upgraded Alifax TEST1 2.0 (Alifax, Polverara, Italy), based on capillary photometry, is designed to improve operator safety and reduce analysis time. This study evaluated its diagnostic performance.

Methods: Precision, comparison, carryover, stability and reference ranges were evaluated in accordance with the International Council for Standardization in Hematology. For comparison with the earlier reference TEST1 1.0, 90 samples from adult and pediatric patients were analyzed in parallel at three ESR measurement ranges (2 - 20, 21 - 60 and 61 - 120 mm/hour) in the analyzer's internal and external modes.

Results: The TEST1 2.0 demonstrated acceptable precision with Alifax latex quality control material and patient samples in the ESR range > 20 mm/hour. The mean ESR was 35.1 ± 32.1 mm/hour versus 35.3 ± 32.4 mm/hour for the reference method ($p = 0.872$), with a mean weighted percentage difference of -0.1 ± 7.6 mm/hour. In the 21 - 58 mm/hour range, the y-intercept was 20.3 (95% CI, 14.7 - 26.0) and the slope 0.5 (95% CI, 0.3 - 0.6), indicating both constant and proportional bias, with a mean difference of -1.8 ± 12.8 mm/hour. ESR measurements on the TEST1 2.0 remained stable for up to 24 hours at room temperature.

Conclusions: In conclusion, the TEST1 2.0 provided rapid and precise ESR measurements, with overall performance comparable to the TEST1 1.0. Differences observed in the clinically significant range highlight the need for careful evaluation and continued performance monitoring in routine laboratory use.

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KEYWORDS

evaluation, ESR, automated analyzer, Test1, alternate method

INTRODUCTION

The erythrocyte sedimentation rate (ESR) is a widely used, non-specific screening test in clinical practice [1]. Although the ESR lacks specificity, it serves as a marker of inflammation, infection, and malignancies [2]. The reference method for measurement of ESR is that proposed by Westergren [3]. This measures the distance in millimeters (mm) that erythrocytes fall in a vertical column after one hour and measures three distinct stages of erythrocyte sedimentation [4]. The ESR measurement reflects a dynamic process of erythrocyte rouleaux for-

mation, due to an increase in positively charged proteins.

The traditional Westergren method has been replaced in most laboratories with alternate and modified automated methods to address the well-documented limitations [5]. Alternative methods for measuring ESR utilize distinct mechanisms such as centrifugation or photometric aggre-gometry [6]. These methods measure the first stage of ESR measurement-rouleaux formation, which results in a faster analysis time. These methods also incorporate advanced features including automated sample mixing, closed tube processing for enhanced operator safety, the use of ethylenediaminetetraacetic acid (EDTA) tubes without the need for dilution, reduced sample volume, and extended sample stability.

Currently, there are several automated ESR analyzers available. The TEST1 (Alifax, Polverara, Italy) is a widely used alternate method for rapid automated ESR analyzer in medium to high volume laboratories [7-11]. More recently, an upgraded version of the analyzer (TEST1 2.0) has been introduced, offering several advantages. These improvements include enhanced correlation with the Westergren method, reduced analysis time per sample (from 60 seconds to 20 - 30 seconds), and increased sample throughput with a capacity of 120 samples per hour. Additional benefits include improved operator safety with its fully closed tube design, an optimized user interface, enhanced internal QC monitoring, and a more compact design [12].

Several studies have evaluated the performance of the older TEST1 analyzer and have consistently demonstrated improved precision [7-11]. This enhanced performance is attributed to the synthetic matrix of its control solution. Unlike other automated ESR methods that use stabilized human blood as control material, TEST1 employs latex-based controls [11]. Compared to the Westergren method, the TEST1 analyzer, which employs a capillary photometric kinetic approach, is less influenced by variations in hematocrit levels [7]. Automated ESR analyzers can serve as reliable alternatives for the gold standard Westergren method [6]). While some discrepancies have been observed between methods, these are largely attributable to differences in methodology [10,13]. To date, however, there are limited published studies evaluating the analytical performance of the upgraded TEST1 2.0 analyzer. The present study therefore aimed to verify the diagnostic utility of the TEST1 2.0 with emphasis on establishing local reference ranges.

MATERIALS AND METHODS

Analyzer

The TEST1 2.0 was installed by the manufacturer over a 5-day period at an academic hospital. The analyzer was calibrated with latex calibrators and the booster Y was aligned with the reference analyzer. Laboratory staff members were trained on instrument operation,

troubleshooting and maintenance procedures.

The TEST1 2.0 is a closed, fully automated analyzer that measures ESR at 37°C. A 150 µL blood sample flows through a capillary tube within the instrument. The diagnostic algorithm transforms the measurement performed within the first 20 seconds into photometric quantitative data. A mathematical algorithm converts the raw data transformed into comparable Westergren values, expressed in mm/hour [12]. The TEST1 2.0 provides a reportable range of 2 to 120 mm/hour. It accommodates EDTA samples in two modes: an internal mode for adult samples and an external mode for pediatric or short samples. The system dimensions are 77 cm in length, 74 cm in width, 86 cm in height, and it weighs 94 kg.

Standards

This verification was performed in accordance with the International Committee on Standardization in Hematology (ICSH) [6] and the method comparison from the Clinical and Laboratory Standards Institute (CLSI) [14]. This study was approved by the Human Research Ethics committee of the University of the Witwatersrand (Protocol number: M2410118).

Samples

The samples used for the verification included latex controls for TEST 1 (3 levels) (Alifax, Polverara, Italy) and residual specimens referred for routine laboratory testing which covered a wide range of normal and abnormal results of clinical relevance. The expected ranges of each level for lot were as follows: 6 - 11, 15 - 22 and 56 - 74 mm/hour. Aged and insufficient samples were excluded. Samples with a hematocrit value < 30% were also excluded. Samples were collected in K2 EDTA or K3EDTA tubes (Vacutainer®, Becton Dickinson, Plymouth, UK) for analysis. Samples were stored at room temperature or 4 - 8°C and analyzed by a dedicated technologist within 12 or 24 hours of collection respectively. Daily QC measurements were performed.

Precision experiment

Within run precision was determined with patient samples and QC material in the normal and abnormal range each analyzed ten times during the same eight-hour period. Between-run precision was determined with QC material in the normal and abnormal range, analyzed three times a day on five consecutive days. This was performed in the internal and external mode. The manufacturer's % CV for low, normal and high QC material were employed as the first hierarchy.

Comparison experiment

Following acceptable routine internal QC procedures, 90 adult and pediatric patient samples were run on the reference instrument (Test1 1.0) followed by the candidate instruments in internal mode and external mode where indicated (insufficient and/or pediatric tube). The new technology was tested over a range of ESR values

of 2 - 120 mm/hour. Samples were tested in three different groups of values: 1 - 20, 21 - 60 and more than 60 mm/hour. The analytical measurement range (2 - 120) was confirmed by determining the highest and lowest measurements with the candidate method.

Method comparison was acceptable if the 95% confidence intervals (CI) for the slopes and intercepts on Deming regression analysis included 1.00 and 0.00, respectively. Correlations and bias were calculated for both the entire analytical range and for the low, middle, and upper third of the analytical range separately. Performance was deemed satisfactory if 95% of the differences between the candidate instrument and the reference ESR were less than ± 8 mm/hour in the range of 2 - 20 mm/hour. For values that exceeded 20 mm/hour the acceptable weighted variation for 120 mm/hour is $\pm 40\%$.

Carryover experiment

Three consecutive analyses of a patient sample with high viscosity (H1, H2, and H3) were analyzed using the Broughton formula. This was immediately followed by three consecutive analyses of a single patient sample with a low viscosity (L1, L2, and L3). This was compared to the manufacturer's specifications (4.2%).

Stability experiment

Stability analysis was performed on five normal and abnormal patient samples. Analysis was performed at time zero (or as close as possible). The samples were kept at room temperature (RT). Subsequent testing was then performed after 6, 12, 18, 24, and 48 hours. The mean percent change from the value at time 0 of the blood specimens stored at RT was calculated and tabulated.

Reference range experiment

Age- and gender-specific reference ranges were determined from at least 80 healthy subjects from the local population. These reference values were compared with the manufacturer's established reference interval. The reference interval was considered valid if $< 10\%$ of the results, within 95% CI, fell outside of the original limits (15).

RESULTS

Precision

The results of the within-run and between-run precision analysis are presented in Tables 1. The within-run and between-run precision analysis at low, middle and high ESR levels was within the manufacturer's limits with QC material. The imprecision of the abnormal low and normal patient samples was high. However, this imprecision is clinically not significant as it covers the normal range. In contrast, the abnormal ESR patient sample which covers the clinically significant ESR range shows an acceptable imprecision of $< 2.7\%$.

Method comparison

The analytical measurement range was 2 - 120 mm/hour on both modes. Differences were acceptable (± 8 mm/hour in the range of 2 - 20) in all samples on the validation Test1 2.0. The mean weighted percentage difference was -0.1 ± 7.6 mm/hour as compared to the reference analyzer. The mean ESR of 35.1 ± 32.1 mm/hour compared with the reference ESR value of 35.3 ± 32.4 mm/hour ($p = 0.872$) (Table 2.). The majority of the data points were within the 95% limits of agreement with few outliers (Figure 1). The outliers have been verified as true measurements performed within 24 hours of collection. On subgroup analysis, in the medium range (21 - 58 mm/hour) the ordinate intercepts and slopes and their corresponding 95% CI indicated a constant and proportionate difference between the two methods. Slightly lower values were obtained with the validation Test1 2.0. The mean weighted percentage difference in the medium range (-0.6 ± 10.7) was, however, acceptable.

Carryover

The percentage carryover was within the manufacturer's specifications on the internal and external modes (0% and 2.3%, respectively) with high and low patient samples.

Stability

The ESR was stable for at least 24 hours after collection when stored at RT (Table 3).

Reference intervals

Reference intervals were established using 80 healthy males and females. The ESR was within the recommended manufacturer's reference intervals (Table 4).

DISCUSSION

Most laboratories globally have transitioned to alternate ESR methods due to their numerous benefits [6]. This study evaluated the bench-top TEST1 2.0 alternate ESR method in an adult and pediatric patient population. This method has been developed for medium to large-sized laboratories. The TEST1 2.0 analyzer offers several advantages that enhance the reliability and efficiency of ESR measurement. The pre-analytical steps of mixing are fully automated. The system maintains a stable internal temperature of 37°C . A single capillary pathway is used for sample flow. Instrument maintenance is minimal [12]. Importantly, TEST1 2.0 uses EDTA-anticoagulated blood without requiring dilution. This eliminates the need for sodium citrate, minimizing pre-analytical errors and improving sample stability. Since introduction, automated ESR methods have consistently demonstrated improved precision, as compared to the manual Westergren method [5,11]. Cennamo et al. reported a strong between-run precision on the TEST1 1.0 using QC material, with CVs of 0%, 3.3%,

Table 1. Precision analysis for normal, abnormal high and low control material and patient samples.

Control samples	Within-run precision control samples CV%			Manufacturer's acceptance criteria for precision CV%		
Parameter (n = 10)	abnormal control low	normal control	abnormal control high	low	normal	high
Test1 2.0 external mode ESR, mm/hour	0.00	0.00	0.69	7.52	3.28	2.70
Test1 2.0 internal mode ESR, mm/hour	0.0	2.79	1.03	7.52	3.28	2.70
Control samples	between-run precision control samples CV%					
Parameter (n = 25)	abnormal control low	normal control	abnormal control high	low	normal	high
Test1 2.0 external mode ESR, mm/hour	3.65	2.27	2.02	7.52	3.28	2.70
Test1 2.0 internal mode ESR, mm/hour	5.31	1.51	1.38	7.52	3.28	2.70
Patient samples	within-run precision patient samples CV%					
Parameter (n = 10)	abnormal low	normal	abnormal high	low	normal	high
Test1 2.0 external mode ESR, mm/hour	24.79	10.76	0.00	7.52	3.28	2.70
Test1 2.0 internal mode ESR, mm/hour	13.98	9.85	0.00	7.52	3.28	2.70

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

Table 2. Performance of Test1 2.0 as compared to the reference analyzer (Test1 1.0).

	n	Correlation coefficient	Range	Deming regression		Bland-Altman statistics		
				intercept (95% CI)	slope (95% CI)	reference mean $\pm$ SD	validation mean $\pm$ SD	bias $\pm$ SD (%)
Overall	90	0.96	2 - 120	0.2 (-2.6 to 3.0)	0.9 (0.9 to 1.1)	35.3 $\pm$ 32.4	35.1 $\pm$ 32.1	-0.4 $\pm$ 9.1
Low range	38	0.92	2 - 20	0.9 (-0.2 to 2.1)	1.0 (0.8 to 1.00)	7.3 $\pm$ 6.8	7.3 $\pm$ 5.9	-0.7 $\pm$ 2.6
Medium range	37	0.75	21 - 58	<u>20.3</u> (14.7 to 26.0)	<u>0.5</u> (0.3 to 0.6)	39.8 $\pm$ 18.5	39.0 $\pm$ 10.3	-1.8 $\pm$ 12.8
2.0 High range	17	0.85	63 - 120	16.1 (-7.2 to 39.3)	0.9 (0.6 to 1.1)	84.5 $\pm$ 24.4	88.8 $\pm$ 21.5	5.2 $\pm$ 13.0

CI: confidence interval, SD: standard deviation.
Underlined line indicates a significant difference.

and 2% for low, medium, and high ESR ranges, respectively 5. The TEST1 1.0 also showed acceptable within-run precision with patient samples, with a mean CV of 9% for ESR values > 20 mm/hour [5]. Similarly, in this study the within-run and between-run precision of the TEST1 2.0 using latex- based QC material was excellent. In addition, we assessed QC procedures using fresh

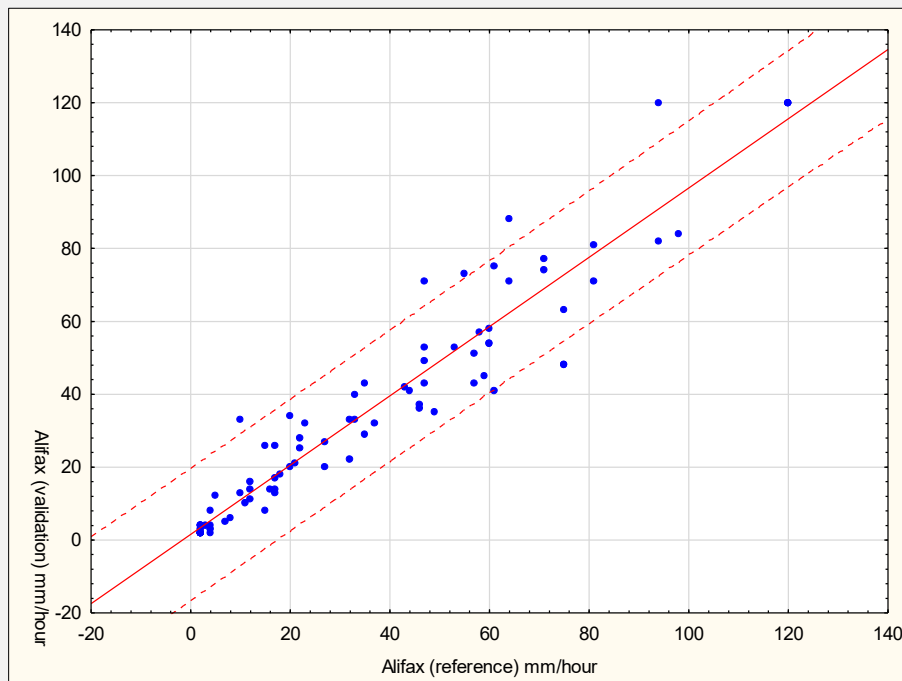
EDTA-anticoagulated patient samples. While the precision of the abnormal low and normal patient samples was high, the precision for ESR values > 20 mm/hour, which covers the clinically significant ESR range, showed an acceptable imprecision of $< 2.7\%$ [2]. In this study, the TEST1 2.0 was compared to the earlier TEST1 1.0 and showed a high correlation ($r = 0.96$, $p <$

Table 3. Stability at room temperature in normal and abnormal samples.

	Storage at room temperature					
	0 h	6 h	12 h	18 h	24 h	48 h
Mean	14.40	14.60	13.40	14.00	13.00	10.60
SD	15.29	14.47	14.91	17.13	16.40	14.42
Mean % difference		1.39	-6.94	-2.78	-1.39	-9.72

Table 4. Reference intervals for ESR for men and women according to age group.

Parameter	n	Lower limit (2.5%), mm/h	Upper limit (97.5%), mm/h	Manufacturer's recommended reference interval mm/h
Total	80	2	31	
Females	40	2	33	
Males	40	2	33	
Males < 50 years	20	2	30	2 - 28
Females < 50 years	20	2	32	2 - 37
Male > 50 years	20	2	31	2 - 37
Female > 50 years	20	2	32	2 - 39

**Figure 1. Deming regression analysis of reference Test1 2.0 as compared to validation Test1 1.0 in the range of 2 - 120 mm/hour.**

0.05). Bland-Altman analysis showed a mean bias of -0.4 ± 9.1 . Nonetheless, in the higher range of clinical interest of > 20 mm/hour there was evidence of a constant and proportionate difference between the two methods and a negative bias for TEST1 2.0 results as compared to TEST1 1.0. Sample instability was not a contributing factor as normal and abnormal patient samples were stable when analyzed on the TEST1 2.0 up to 24 hours when stored at RT. A significant limitation of the Westergren ESR is its requirement for sample analysis within four hours of collection if kept at room temperature, or within twelve hours if refrigerated at $4 - 8^{\circ}\text{C}$ [14]. This restriction often leads to routine rejection of many referred ESR samples. Comparable to other alternative ESR methodologies, the TEST1 2.0 offered enhanced sample stability, allowing for reliable testing up to 24 hours at room temperature [10].

Previous studies have demonstrated systematic biases in automated ESR analyzers based on aggregation or centrifugation principles when compared with the traditional sedimentation-based Westergren method [13,17]. Van der Maas et al. demonstrated a correlation of 0.67 and a mean bias of -4.4 mm/hour (95% CI: -7.8 to 1.0) when comparing the Alifax Roller Test-ITH alternate method to the automated StaRRsed Westergren method (Mechatronics, Zwaag, the Netherlands) [17]. This study further showed that the use of an alternate ESR method impacted the validity of the Disease Activity Score 28 (DAS28). In this study involving patients with rheumatoid arthritis, 26% were misclassified using the Westergren ESR [17]. In another study, the TEST1 1.0 analyzer showed an overall strong correlation with the Westergren method in 680 patients with various rheumatic diseases ($r = 0.90$, $p < 0.001$) (7). Nonetheless, in the higher range of clinical interest of > 20 mm/hour there was evidence of considerable variation and a negative bias for TEST1 1.0 results as compared to Westergren ESR values [7]. Studies, however, comparing alternate and modified automated ESR methods have demonstrated improved agreement. No statistically significant difference was observed in a study involving 245 patient samples when comparing TEST1 1.0 with the Ves-Matic Cube 200, a modified ESR method [18]. Bland-Altman analysis showed a mean bias of -1.1 (95% CI: -1.8 to -0.4), with an upper limit of agreement at 9.8 and a lower limit at -11.9 [18].

According to the ICSH recommendations, laboratories should establish reference intervals for new ESR measurement methods [6]. In this study, the ESR values were within the manufacturer's recommended reference intervals. Population-specific differences may account for variation in reference values across studies. The reference intervals obtained in the present study were similar to those reported in an Italian population ($2 - 28$ mm/hour in men and $2 - 37$ mm/hour in women, aged $15 - 50$ years) [19], but lower than those observed in a Korean population ($2 - 19$ mm/hour in men and $2 - 26$ mm/hour in women) [20]. It is therefore important to advise clinicians of any changes in reference intervals,

and to provide an appropriate interpretative comment to ensure correct clinical interpretation.

A limitation of this study is that the TEST1 2.0 was not compared with the manual Westergren method which is the gold standard. Previous studies have reported a significant mean difference between the alternate ESR and Westergren ESR methods [13,17]. Westergren ESR testing methods are influenced by intrinsic factors such as hematocrit and viscosity as well as extrinsic factors such as sample length and temperature whereas these factors do not interfere significantly with ESR measurement on alternate ESR methods. Nonetheless, this study adds to the existing body of research, highlighting the global shift from the traditional Westergren method to automated alternate or modified methods due to its inherent limitations.

In conclusion, the TEST1 2.0 is an alternative method for ESR measurement that provides rapid and precise results. While overall performance was comparable to the earlier TEST1 1.0 for a wide range of adult and pediatric samples in the internal and external modes, respectively, differences were noted in the analytical range of $21 - 58$ mm/hour. Careful evaluation and ongoing performance monitoring are therefore recommended when implementing these novel methods in routine laboratory practice.

Declaration of Generative AI in Scientific Writing:

The authors declare that no generative AI tools were used in the preparation of this manuscript. All authors agree to the submission.

Declaration of Interest:

The author(s) declare no conflict of interests with respect to the authorship and/or publication of this article. The authors declare the manuscript has not been published in whole or in parts nor submitted elsewhere.

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