Received: 15 August 2017 | Accepted: 18 December 2017

DOI: 10.1002/jcla.22384

RESEARCH ARTICLE

WILEY

Test-1 analyzer and conventional Westergren method for erythrocyte sedimentation rate: A comparative study between two laboratories

Cigdem Sonmez[1] | Ozlem Ceylan Dogan[2] | Aysegul Ozturk Kaymak[3] | Nedim Akkaya[1] | Kadir Okhan Akin[4] | Gulcan Guntas[5]

[1]Central Laboratory, Dr. Abdurrahman Yurtaslan Oncology Education and Research Hospital, Ankara, Turkey

[2]Faculty Of Medicine, Ibni Sina Hospital, Central Laboratory, Ankara University, Ankara, Turkey

[3]Genetics Laboratory, Dr. Abdurrahman Yurtaslan Oncology Education and Research Hospital, Ankara, Turkey

[4]Central Laboratory, Ankara Medical Park Hospital, Ankara, Turkey

[5]Biochemistry Department, Kırklareli University School of Health, Kırklareli, Turkey

Correspondence
Cigdem Sönmez, Dr. Abdurrahman Yurtarslan Demetevler Oncology, Education and Research Hospital, Ankara, Turkey.
Email: dr.csonmez@gmail.com

Background: Measurement of the length of sedimentation reaction in blood (LSRB), also called erythrocyte sedimentation rate (ESR), is a widely used hematology test. This study intends to compare ESR levels measured by Test-1 method and International Council for Standardization in Hematology's (ICSH) reference method, and analyzes the effect of hematocrit (Hct) on ESR results.

Material and Methods: A total of 755 patients from 2 hospitals were included in the study, and samples with EDTA were studied by Test-1 method for ESR measurement and total blood count, whereas citrated samples were studied with reference Westergren method. Then, 2 methods were compared. Distribution of ESR results according to the ESR(≤20, >20 mm/h) and Hct(≥35%, <35%) levels and hospital type was analyzed. ESR levels with Hct levels<35% were corrected with Fabry's formula.

Results: The mean and SD values for the Test-1 method, reference Westergren method, and corrected ESR measurement were 21.30 ± 18.39, 28.59 ± 25.82, and 24.92 ± 20.58 mm/h, respectively. Within the whole group, the correlation coefficient (*r*) was .77 (.7-.80) with a significance level $P < .001$. Passing-Bablok regression analysis of the methods resulted in a regression equation y = 1.00 (95% CI: 0.43-1.88) + 0.75 (95% CI: 0.70-0.78)x while the significance of linearity was acceptable ($P < .01$). All subgroup linear regression analyses revealed that the correlation was acceptable, except ESR > 20 mm/h group, Hct < 35% group, and corrected ESR group (significance level were $P > .10$).

Conclusion: The study showed that the role of the hospital and the capacity of testing are important in choosing the instrument for measuring ESR. Furthermore, the patient profile, especially malignancy possibility and Hct level, may be important for instrument selection.

KEYWORDS

erythrocyte sedimentation rate, Fabry's formula, hematocrit, Test-1 method, Westergren method

1 | INTRODUCTION

Measurement of the length of sedimentation reaction in blood (LSRB), also called erythrocyte sedimentation rate (ESR), is a common, cheap, widely used, and practical hematology test.[1,2] Despite the widespread use of ESR in clinical practice, it is a nonspecific test parameter which increases in several disease groups, especially in infection, inflammatory diseases, and malignancy.[1,3,4] Although ESR is a nonspecific test,

its level is important for diagnosis and follow-up of rheumatoid arthritis, temporal arteritis, and polymyalgia rheumatic disease.[4-7] Higher ESR levels are also found in anemia and with increased fibrinogen, α-2 macroglobulin, and plasma protein (Immunoglobulin M) levels, and its level is affected by physiological conditions such as age, gender, and the possibility of pregnancy.[8,9]

Red blood cells' sediment in a period of 1 hour has 3 phases. The first phase is falling of the single red blood cells and then red cells' forming stacks called rouleaux, which settle faster. The second phase is falling of rouleaux and aggregates, and the last step is cell packing. When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other.[4,8,9] To analyze ESR, International Council for Standardization in Hematology (ICSH) recommends the Westergren method as the reference method.[10,11] To perform the test, anticoagulated blood is traditionally placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall in an hour is measured and reported in mm/h.

The Westergren method is not practical in clinical laboratory as it takes long to run, needs large volumes of specimen, and has safety risks. In recent years, several new techniques which use different methods and sample types to measure ESR have been developed. Since the introduction of automated analyzers into clinical laboratory, the ESR test has been automatically performed. These close systems were safer for the laboratory operators and allowed for studying with 1 type of sample in several systems. They also decreased the amount of blood sample that is taken from patients.[7,12] One of these systems is Test-1 method, which studies with photometric method and uses samples with EDTA.

This study aims to compare the ESR levels measured with Test-1 method and ISCH's reference method (Westergren). It also investigates the higher ESR results measured by the 2 methods and the effect of hematocrit (Hct) levels on ESR results.

2 | MATERIALS AND METHODS

2.1 | Subjects and blood samples

Patients, who had the ESR and complete blood count test request, were scheduled for the study. After taking the informed consent from the patients, blood was collected in 3-mL EDTA tubes (K2E 5.4 mg, BD Vacutainer, Plymouth, UK) and citrated tubes (0.105 mol/L sodium citrate; 1 BD Vacutainer® Seditainer™ Glass tube with Black Conventional Closure) under standardized conditions. This study was approved by Kecioren Education and Research Hospital Ethics Committee. The blood samples were obtained from 2 hospitals (Kecioren Education and Research Hospital [Hospital A] and Dr Abdurrahman Yurtarslan Oncology Education and Research Hospital [Hospital B]). The blood samples were analyzed in their own Clinical Chemistry Laboratories. Hospital A is a general hospital which has 300 beds for inpatients and 1 120 000 polyclinics per year for outpatients. Hospital B has 600 beds and is equipped to treat medical, surgical oncology, and bone marrow transplantation patients. Totally, 833 samples were collected from the patients, but 78 were rejected due to the clotting. Pregnant patients and patients under age 18 were not included in the study. A total of 275 patient samples were collected from Hospital B and 480 were from Hospital A.

In both hospitals, samples with EDTA were studied by Test-1 method (Alifax; Test-1-THL, Padova, Italy). The complete blood counts were performed by hematology analyzers in Hospital B (Advia Centaur 2120; Siemens, Munich, Germany) and in Hospital A (LH 780; Beckman Coulter, Miami, USA). Citrated samples were studied with reference Westergren method (BD SeditainerTM Manual ESR Stand; BD).

Totally, 755 paired blood samples were compared with each other, and the ESR results distributed as follows:

- According to ESR levels, the samples were divided into 2 groups: 395 samples in Group I with ESR levels ≤20 mm/h, and 360 samples in Group II with ESR levels >20 mm/h. Both subgroups were compared with each other to determine the effect of the ESR level on the method type.
- According to Hct levels, samples were divided into 2 groups: 624 samples in Group I with Hct levels ≥35%, and 131 samples in Group II with ESR levels <35%. ESR levels were corrected using Fabry's formula (corrected ESR = measured ESR × 15 / [55-Hct]) for the samples whose Hct levels were <35% (Group II).[8] Statistical analyses were performed after required corrections.
- According to hospital (2 groups), Hospital A had 480 samples while Hospital B had 275 blood samples.

2.2 | Method descriptions

2.2.1 | Reference Westergren method

The citrated blood was mixed manually. Samples were placed into the sedimentation measurement stand (BD Seditainer™ Manual ESR BD) according to the ICHS's guideline recommendations, which has 200-mm scale. One hour later, ESR was measured in mm. When the Hct level <35%, Fabry's formula was used. One hundred and thirty-one ESR levels, which had Hct level <35%, were corrected with this formula.

2.2.2 | Test-1 method

Blood samples with EDTA were studied in Test-1 device according to the instruction. The blood samples were mixed slowly for 120 seconds; then, 150 μL of blood samples was transferred to the capillaries that are kept at 37°C. Aggregation and sedimentation capacity of erythrocytes were measured photometrically at 950-nm wavelength.[13]

2.3 | Statistics

The results were statistically analyzed using SPPS version 13.0 software (Chicago, IL, USA). Summary statistics of each parameter were reported in mean ± standard deviation (SD). The Pearson test was

TABLE 1 Evaluation of ESR measurement mean and SD values for the Test-1 method, reference Westergren method, and corrected ESR measurement

Parameter	Mean ± SD	95% CI
Test-1 method (mm/h)	21.30 ± 18.39	19.99-22.62
Westergren method (mm/h)	28.59 ± 25.82	26.74-30.43
Corrected ESR (mm/h)	24.92 ± 20.58	23.45-26.39

%, percentage; SD, standard deviation; CI, confidence interval; ESR, erythrocyte sedimentation rate.
Values were given as mean ± SD with 95% CI. Paired *t* test ($P < .05$) revealed no significant differences between the groups.

used for the correlation. Passing-Bablok linear regression analysis was used to compare ESR values, and Bland-Altman analysis was also performed to evaluate bias and 95% CI limits of agreement.[14] Differences between dependent groups were examined with paired *t* test, where statistical significance level was accepted as $P < .05$.

3 | RESULTS

Of the 755 patients (mean age 50.27 ± 16.9 years old), 363 were men and 391 were women. The mean age of men and women were 48.84 ± 16.65 and 51.53 ± 17.09, respectively. The overall mean Hct level was 40.06 ± 5.96%, while Hct levels of 131 samples were <35% (mean ± SD levels: 30.47 ± 4.06%); the Hct levels of the remaining 624 ESR samples were ≥35% (mean ± SD levels: 42.07 ± 4.05%). Hct levels of samples (480 patients) obtained from Hospital A and B were 41.62 ± 5.12% and 37.32 ± 6%, respectively. The mean ESR levels measured by each method were given in Table 1. As regards the method used, Paired *t* test ($P < .05$) revealed no significant differences between the groups. Figure 1 presents the box-and-whisker graphic distribution of each method. ESR Levels with Westergren method were higher than those with the Test-1 method. However, corrected ESR levels were lowered with Fabry's formula and getting similar to Test-1 method (Figure 1).

Within the whole group, the correlations were comparable and correlated with each other. The correlation coefficient (*r*) was .77 (.74-.80) with a significance level $P < .0001$ (Figure 2). Passing-Bablok regression analysis between methods resulted in a regression equation y = 1.00 (95% CI: 0.43-1.88) + 0.75x (95% CI: 0.70-0.78), and the significance of linearity was acceptable ($P < .01$). The Bland-Altman plots between 2 groups were shown in Figure 3. Bland-Altman data analysis showed no systemic bias, and 95% of all samples fell into the limit of agreement.

Owing to the factors influencing the results of ESR, a subgroup analysis was performed, in which 755 samples were created according to the methods, ESR levels (ESR ≤ 20 mm/h, ESR > 20 mm/h), Hct levels (Hct ≥ 35%, Hct < 35%), and hospital role. Method comparison results of the whole group and subgroups are shown in Table 2. An analysis of the subgroups obtained from ESR levels demonstrates that although Test-1 ESR levels (mean ± SD 9.68 ± 6.58 mm/h) were lower than those of the Westergren method (mean ± SD 9.10 ± 5.51 mm/h), there is good concordance with respect to clinical interpretation in ESR ≤ 20 mm/h ($r = .61$, $P < .01$). A lower correlation was seen in the ESR>20 mm/h group ($r = .56$, $P > .10$). Between these 2 groups, the lowest difference was seen in the group that had ESR levels ≤20 mm/h ($r = .61$, $P < .01$).

Investigation of the ESR results according to Hct level showed that, in the Hct < 35% group, the mean value of samples with Westergren method, Test-1 method, and corrected ESR was 54.084 ± 31.6, 35.41 ± 22.91, and 33.05 ± 17.7 mm/h, respectively. On the other hand, the mean ESR levels for Westergren method and Test-1 method were 23.42 ± 20.73 and 18.34 ± 15.79 mm/h, respectively in the Hct ≥ 35% group. For these 3 groups, the number of samples, correlation coefficients, and method comparison results are shown in Table 2. After applying Fabry's formula, corrected ESR group's correlation was getting strong, but the significance level was still unacceptable ($r = .54$, $P > .10$). Hct < 35% group showed a poor agreement between the 2 methods with a slope of the Passing-Bablok curve similar to ESR > 20 mm/h ($P > .10$) (Table 2).

The ESR results according to hospital type revealed that, in Hospital A, mean ESR levels with Westergren method and Test-1 method were 23.86 ± 21.92 and 21.08 ± 17.25 mm/h, respectively. The mean ESR levels with Westergren method and Test-1 method, in Hospital B, were 36.85 ± 29.78 and 21.70 ± 20.26 mm/h, respectively. Table 2 presents the number of samples, correlation coefficients, and method comparison results pertaining to the 2 hospitals.

In all groups, the strongest correlation was seen in Hospital A ($r = .85$, $P < .01$), while the weakest correlation was in the Hct < 35% group($r = .54$, $P > .10$). Although Test-1 ESR readings were on average lower than those of the Westergren method, a good concordance with respect to clinical interpretation was found (Table 2, Figures 1 and 2) More particularly, discordant results were only found when ESR readings were high in combination with corrected ESR (Hct < 35%). In the linear regression analysis, these 2 groups' (ESR > 20 mm/h, Hct < 35%) significance levels were unacceptable ($P > .10$).

4 | DISCUSSION

ESR is a simple, inexpensive, and practical test which is commonly used all over the world in the diagnosis and follow-up of inflammatory diseases, infection, and malignancy. Although this test has high sensitivity, its specificity is low.[15] ESR test result is affected by the red blood cell concentration, hematocrit level, plasma viscosity, and plasma proteins including fibrinogen, albumin, and globulins.[2,4,8,16] Although ICSH suggests Westergren method as a reference method for ESR, this method has disadvantages as to the low hematocrit levels, and needs longer time and large amount of specimen to run.[2,4] As the Westergren method overestimates ESR in samples with low Hct, using Fabry's formula has been recommended to correct ESR measurement.[2,8]

With technological innovations, Test-1 generates fast, reliable results and uses the EDTA samples to measure the ESR levels. This

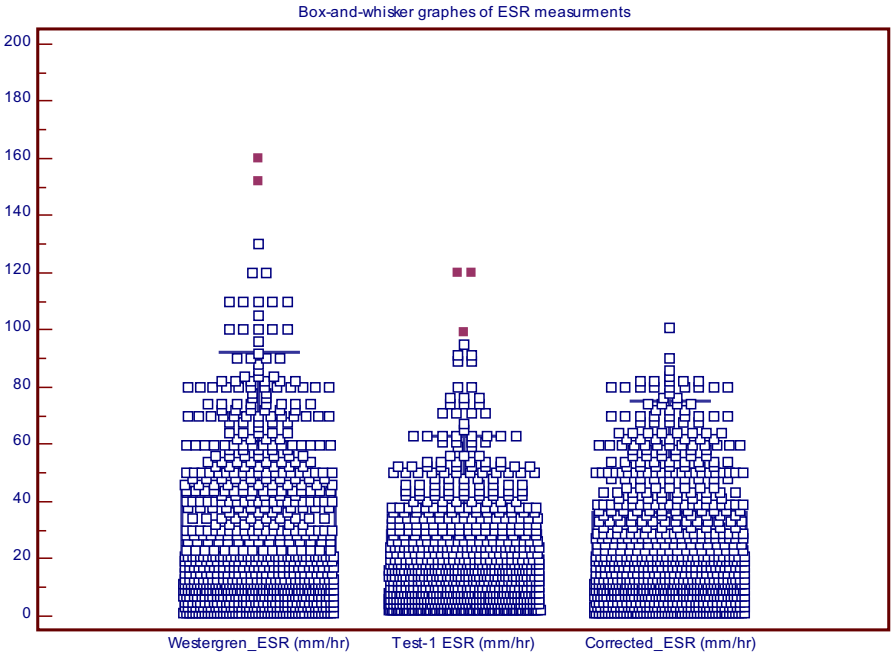

FIGURE 1 Box-and-whisker graph of all erythrocyte sedimentation rate (ESR) levels obtained from Westergren method, Test-1 method, and corrected ESR levels with Fabry's formula

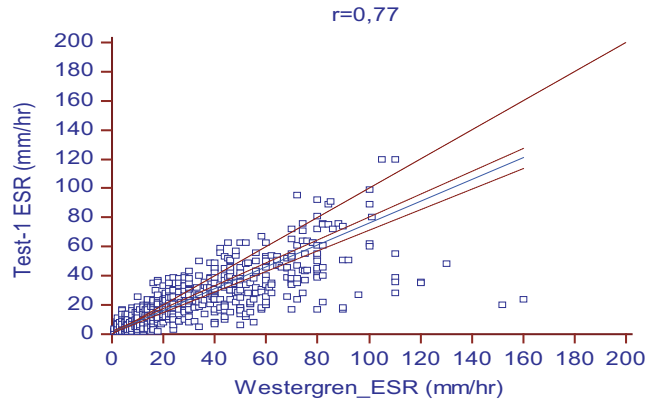

FIGURE 2 Overall linear regression graphs

system has another advantage in that it is not influenced by the hematocrit level, plasma viscosity, and plasma proteins,[16] whereas at higher ESR levels, the Test-1 instrument showed slight deviation from the reference method.[16,17]

The present study pointed to a good correlation between the Test-1 instrument and the reference Westergren ICHS method ESR measurements. Recent studies also found similar results about the differences between the 2 methods.[2,4,7,18] Similarly, they showed the Test-1 ESR values were lower than the Westergren values. This study also showed that with the low Hct (<35%), ESR levels were higher with the reference Westergren method and showed poor correlation as in previous studies.[2] After applying Fabry's formula, the corrected Westergren ESR results turned out to have a stronger correlation with the Test-1 results, but still, the significance value *P* was >.10. These findings are similar to those of studies conducted by Cha et al[18] and Romero et al[2] These researchers, similarly, found that the differences between Test-1 and corrected Westergren values were smaller than differences between TEST 1 and noncorrected Westergren values.

When the entire group was divided according to the Westergren ESR levels, poor correlation with Test-1 was observed(r = .61, P > .10) with the higher ESR results (>20 mm/h). Moreover, with regression analysis, the Test-1 instrument results showed negative deviation. Haderman et al also published the possibility of the low results with Test-1 device due to the incomplete disaggregation at the start of the measurement for the higher ESR levels.[16,17] In these studies, they reported that, in order to avoid this condition, before studying with the Test-1 instrument, mixing the specimen is important to obtain accurate results.[16,17] Romero et al found that the ESR levels over 55 mm/h. had no significant bias, but the limit of agreement was too wide for the clinical acceptance and suggested that, at higher ESR levels, these 2 methods cannot be used interchangeably.[2] In the present study, the correlation significance level was *P* > .10 in the group that had ESR levels >20 mm/h group.

When 2 hospitals were compared, the correlation of ESR results in Hospital B (oncology hospital) was poor as expected, due to higher ESR and lower Hct levels because most patients had malignancy in this hospital. After correction of Westergren ESR results with Fabry's formula, better correlation was observed within the hospitals according to Hct level. Cha et al found similar results with 189 samples and 3 Test-1 devices in 3 hospitals when they did comparison according to the reference Westergren method.[18] Our findings are in accordance with Cha et al's study as Test-1 instrument proved more suitable than Westergren method in patients with malignancy. On the other hand, in another study involving 680 patients, Haderman et al[16] reported a slight deviation at higher ESR levels (60 m/h) as also seen in our study. Thus, while using Test-1 instrument, operators should be aware of deviation at high ESR levels due to the disaggregation and the short duration time period of measurement.[2,16,17] Cha et al found that ESR levels with Westergren

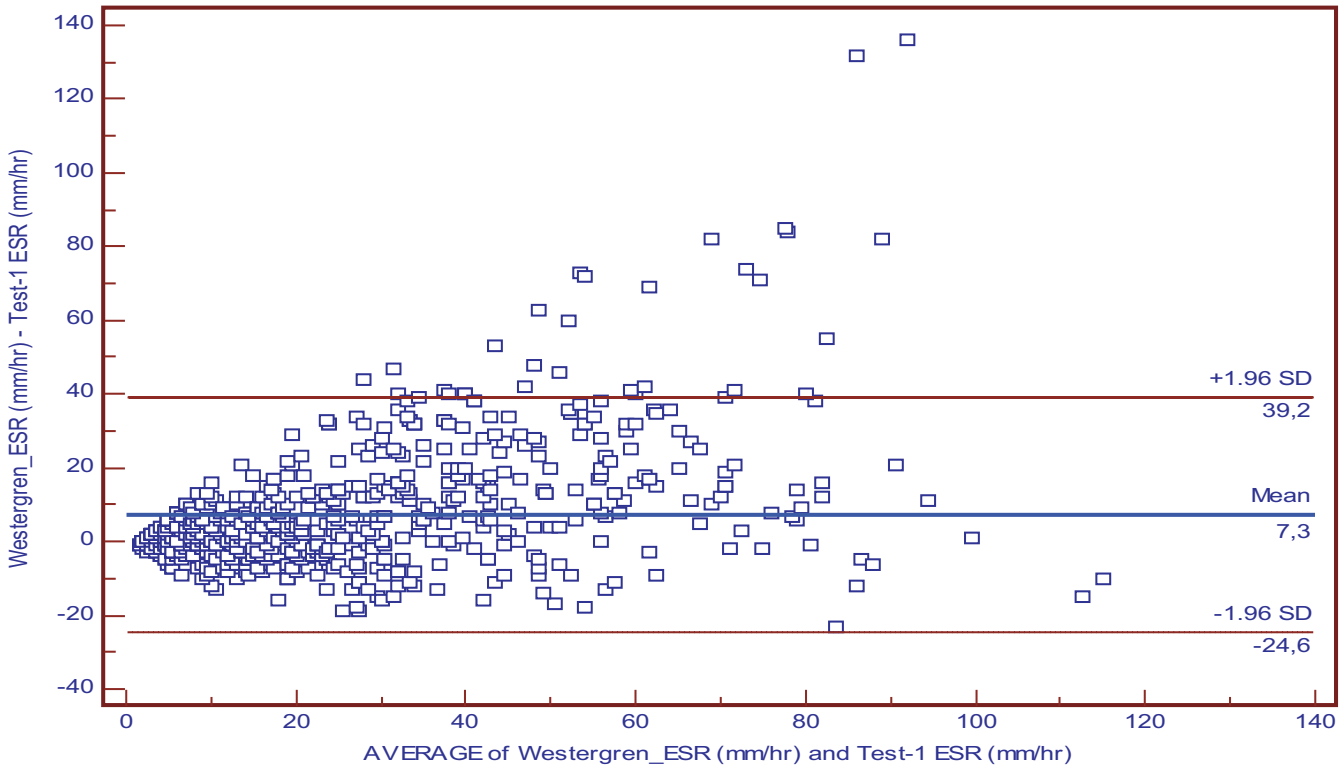

FIGURE 3 Bland-Altman plot of total erythrocyte sedimentation rate levels with Westergren method and Test-1 method. In the group, a good agreement between the Westergren method (x) and the TEST 1 (y) was found with a regression equation of the Passing-Bablok method comparison of y = 1.00 + 0.75x while the significance of linearity was acceptable ($P < .01$). (95% confidence interval [CI] slope was 0.43-1.88 and intercept was 0.70-0.78)

method were higher than Test-1 method, especially in samples with Hct < 35%. Cha et al and Romeola stated that Westergren method overestimates ESR in samples of low Hct.[2,18]

In conclusion, the role of the hospital and the capacity of testing are important in choosing the instrument to measure ESR. In addition, patient profile especially possibility of malignancy and Hct level may be critical for the instrument selection. For emergency and routine clinical laboratories that have huge workload, Test-1 instrument will be a suitable choice, provided that the deviation of higher ESR is considered. On the other hand, the reference Westergren method is suitable

TABLE 2 Comparison of erythrocyte sedimentation rate (ESR) measured with Westergren method and Test-1 method and subgroups (ESR [≤20, >20 mm/h], Hct [<35%, ≥35%] levels, and hospital type [Hospital A-B])

Group	n	*r*	Bias	95% CI	Difference	Limits of agreement	*P*
Total	755	.77	1	0.43-1.88	7.3	24.6-39.2	(<.01)
ESR ≤ 20 (mm/h)	395	.61	−0.88	0.00-2.00	0.4	−10.3-11	(<.01)
ESR > 20 (mm/h)	360	.56	−3.14	1.30-6.50	14.9	−25-54.7	**(>.10)**
ESR with Hct < 35%	131	.54	−0.64	4.93-5.21	18.7	34.6-71.9	**(>.10)**
Corrected ESR With Hct < 35%	131	.64	−2.22	1-8.20	7.5	−26.6-41.5	**(>.10)**
ESR with Hct ≥ 35%	624	.83	1.03	0.35-1.50	4.9	−17.8-27.6	(<.01)
Hospital A	480	.85	1.38	0.95-2.20	25.6	−67.4-16.2	(<.01)
Hospital B	275	.74	−0.75	−1.92-0.57	15.2	−25-54.3	(<.05)

P value of <.05 was considered statistically significant.
Bold values mean statistical significance.
The table shows the number of the data, correlation, bias, difference and limit of agreement, and the significance level.
In all groups, the strongest correlation was seen in Hospital A, while the weakest correlation was in the Hct <35% group. Discordant results were found in these two groups' (ESR>20mm/hr, Hct<35%) significance levels were unacceptable. After applying Fabry's Formula, Corrected ESR group's correlation was still unacceptable.

for other laboratories. As the low Hct has the risk of overestimating the ESR measurement with reference Westergren method, correcting the ESR results with Fabry's formula should not be neglected.

COMPLIANCE WITH ETHICAL STANDARDS

All authors have read and agree to the publication of the article and that the article has not been submitted elsewhere.

This study was approved by Kecioren Education and Research Hospital Ethics Committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

ORCID

Cigdem Sonmez http://orcid.org/0000-0001-9307-5674
Ozlem Ceylan Dogan http://orcid.org/0000-0003-3078-999X

REFERENCES

1. Osei-Bimpong A, Meek JH, Lewis SM. ESR or CRP? A comparison of their clinical utility. *Hematology*. 2007;12:353-357.
2. Romero A, Muñoz M, Ramírez G. Length of sedimentation reaction in blood: a comparison of the test 1 ESR system with the ICSH reference method and the sedisystem 15. *Clin Chem Lab Med*. 2003;41:232-237.
3. Mahlangu JN, Davids M. Three-way comparison of methods for the measurement of the erythrocyte sedimentation rate. *J Clin Lab Anal*. 2008;22:346-352.
4. Arikan S, Akalin N. Comparison of the erythrocyte sedimentation rate measured by the Micro Test 1 sedimentation analyzer and the conventional Westergren method. *Ann Saudi Med*. 2007;27:362-365.
5. Sezer S, Yilmaz FM, Kaya O, Uysal S. Evaluation of Ves-Matic Cube 200 for erythrocyte sedimentation rate determination. *J Clin Lab Anal*. 2013;27:367-372.
6. Curvers J, Kooren J, Laan M, et al. Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergren-based methods. *Am J Clin Pathol*. 2010;134:653-660.
7. Plebani M, Piva E. Erythrocyte sedimentation rate: use of fresh blood for quality control. *Am J Clin Pathol*. 2002;117:621-626.
8. Fabry TL. Mechanism of erythrocyte aggregation and sedimentation. *Blood*. 1987;70:1572-1576.
9. Hansson LO, Carlsson I, Hansson E, Hovelius B, Svensson P, Tryding N. Measurement of C-reactive protein and the erythrocyte sedimentation rate in general practice. *Scand J Prim Health Care*. 1995;13:39-45.
10. Vennapusa B, De La Cruz L, Shah H, Michalski V, Zhang QY. Erythrocyte sedimentation rate (ESR) measured by the Streck ESR-Auto Plus is higher than with the Sediplast Westergren method: a validation study. *Am J Clin Pathol*. 2011;135:386-390.
11. Jou JM, Lewis SM, Briggs C, et al. ICSH review of the measurement of the erythocyte sedimentation rate. *Int J Lab Hematol*. 2011;33:125-132.
12. Atas A, Cakmak A, Soran M, Karazeybek H. Comparative study between the Ves-matic and microerythrocyte sedimentation rate method. *J Clin Lab Anal*. 2008;22:70-72.
13. Plebani M, De Toni S, Sanzari MC, Bernard D, Stockrelter E. The TEST 1 automated system: a new method for measuring the erythrocyte sedimentation rate. *Am J Clin Pathol*. 1998;110:334-340.
14. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry. Part I. *J Clin Chem Clin Biochem*. 1983;21:709-720.
15. Dreyer SJ, Boden SD. Laboratory evaluation in neck pain. *Phys Med Rehabil Clin N Am*. 2003;14:589-604. Review
16. Hardeman MR, Levitus M, Pelliccia A, Bouman AA. Test 1 analyser for determination of ESR. 1. Practical evaluation and comparison with the Westergren technique. *Scand J Clin Lab Invest*. 2010;70:21-25.
17. Hardeman MR, Levitus M, Pelliccia A, Bouman AA. Test 1 analyser for determination of ESR. 2. Experimental evaluation and comparison with RBC aggregometry. *Scand J Clin Lab Invest*. 2010;70:26-32.
18. Cha CH, Cha YJ, Park CJ, et al. Evaluation of the TEST 1 erythrocyte sedimentation rate system and intra- and inter-laboratory quality control using new latex control materials. *Clin Chem Lab Med*. 2010;48:1043-1048.

How to cite this article: Sonmez C, Dogan OC, Kaymak AO, Akkaya N, Akin KO, Guntas G. Test-1 analyzer and conventional Westergren method for erythrocyte sedimentation rate: A comparative study between two laboratories. *J Clin Lab Anal*. 2018;32:e22384. https://doi.org/10.1002/jcla.22384