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Research Article

Evaluation of the Vision C erythrocyte sedimentation rate analyzer

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Abstract

Objectives: The International Council for Standardization in Haematology recommends that new methods and instruments used in medical laboratories be thoroughly tested with an analytical performance evaluation prior to implementation. This study is an assessment of the analytical performance characteristics of the Vision C analyzer (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China), which uses a modified Westergren method to measure erythrocyte sedimentation rate (ESR) and a comparison with the reference Westergren method.

Methods: The analytical performance of the Vision C was evaluated according to intra-run/inter-run precision, stability, and compatibility with the gold standard Westergren method. In all, 173 patient samples were studied using both methods. The Westergren method levels were used to form 3 groups: <40, 40-80, and >80 mm/hour.

Results: The intra-run precision ranged from 4.93% to 18.18% for 5 samples at different levels. Inter-run precision for the normal level was 7.03% and 2.94% for the pathological level. Both the room temperature and refrigerated samples were stable at 4 hours. The overall bias between the Westergren method and the Vision C analyzer was -5.23 mm/hour (confidence interval [CI]: -6.66 to -3.79), the correlation coefficient was 0.948, and the Passing-Bablok regression equation was y=2.073+0.805x. The bias of the Vision C for ESR values <40 mm/hour was -0.885 (CI: -2.027 to 0.258), -9.23 (CI: -11.853 to -6.613) for values 40-80 mm/hour, and -17.26 (CI: -21.306 to -13.227) for values >80 mm/hour.

Conclusion: The Vision C analyzer using a modified Westergren method met the precision and sample stability criteria, but the results were not fully compatible with the Westergren method at all ESR levels, and the difference was significant at high ESR levels.

Keywords: Erythrocyte sedimentation rate, modified Westergren, Vision C analyzer, Westergren

Erythrocyte sedimentation rate (ESR) is a traditional laboratory test that has been used for many years to evaluate the presence of inflammation. According to the classic definition, ESR is the measurement of the vertical fall (in millimeters) of the red blood cells in plasma in a tube over a 1-hour period. The ESR increases in many different conditions, such as inflammation, infection, tissue damage, autoimmune diseases, and malignancy [1]. The ESR value is commonly used in the diagnosis of rheumatological diseases, such as rheumatoid arthritis, temporal arteritis, and polymyalgia rheumatica [2-4], in the follow-up of orthopedic infections [5], and as a prognostic factor for Hodgkin lymphoma [6].

The Westergren ESR measurement method was developed by Dr. Alf Vilhelm Albertsson Westergren in 1921 [7]. It uses the whole blood sample diluted with citrate, and was accepted as the reference ESR measurement method by the International Council for Standardization in Haematology (ICSH) in 1973 [8]. This method is still the ICSH reference ESR measurement method [9] and standard used in the H02-A5/Erythrocyte Sedimentation Rate Measurement Procedures published by the Clinical Laboratory Standards Institute (CLSI) [10].

In the last 20 years, new instruments have been developed that use different ESR methods. The current ESR measurement methods have been divided into 3 categories by the

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ICSH ESR Working Group: standard Westergren, modified Westergren, and alternative methods. The standard Westergren method dilutes blood with trisodium citrate dihydrate (1:4) and reports precipitation after 60 minutes in mm. Modified Westergren methods are based on the Westergren method, but have some modifications, such as reduced measurement time and undiluted measurement using a different anticoagulant (ethylenediaminetetraacetic acid [EDTA]). Alternative ESR methods do not rely on the Westergren method and use new approaches, such as centrifugation and photometric rheology [9, 11].

The disadvantages of the traditional Westergren method are a longer measurement time (60 minutes), dilution of blood with citrate, and the potential for human error with laboratory automation systems. Advantages of modified and alternative ESR methods include a shorter working time, reduced exposure of laboratory personnel to a potentially infectious agent, reduced cost due to the opportunity to use the same tube containing EDTA that is used for a complete blood count analysis, and the automatic transfer of results to the laboratory information system, which decreases human error due to manual transcription of results [11].

Instrument and method changes are common in medical laboratories. However, it is important to assess the compatibility of the new method with the reference method or the existing method in use. This study was designed to evaluate the analytical performance of the Vision C ESR analyzer (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China), which uses a modified Westergren method, prior to use in our laboratory.

Materials and Methods

Ethics approval for this study was granted by the Suleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee on May 7, 2019 (no: 154).

An analytical performance evaluation of the Vision C analyzer was performed in the medical biochemistry laboratory of the Suleyman Demirel University Research and Practice Hospital. Intra-run precision, inter-run precision, stability, and method comparison studies were performed using the Westergren method according to ICSH recommendations for modified and alternate methods to measure ESR [11].

Blood samples

A total of 173 blood samples collected in tubes containing EDTA (BD Vacutainer K2EDTA blood collection tubes, 2 mL, 13x75 mm; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and transported to the clinical biochemistry laboratory via a pneumatic tube transport system within a maximum of 15 minutes were used to assess the analytical performance of the Vision C (intra-day and inter-run precision, sample stability, and method comparison studies). All of the blood samples included in the study were clot-free, contained 2 mL of content in the tube, and had hematocrit values within the reference ranges. To conduct the method comparison, the ESR measurements were first completed using the Vision C instrument; ESR measurements were subsequently performed using the standard Westergren method.

Description of Vision C instrument method

The Vision C analyzer, which uses a modified Westergren method, has the capacity for 32 samples and can determine the ESR in 20 minutes with 120 infrared readings performed every 10 seconds after gently mixing the EDTA tubes at 180°. At actual room temperature, the instrument extrapolates the ESR result in 20 to 60 minutes. It can then report the results with a correction to the standard temperature of 18ºC according to Manley's nomogram [12].

Precision study of Vision C instrument

Intra-run precision testing was conducted using the ESR measurement of 5 randomly selected samples covering the analytical measurement range and repeated 10 times during an 8-hour period. For inter-run precision, 2 levels of control (Bio-Rad Liquicheck Sedimentation Rate Control, Level 1, lot number: 24381, and Level 2, lot number: 24382; Bio-Rad Laboratories Inc., Hercules, CA, USA) were studied 3 times a day for 5 consecutive days. The mean, SD, and coefficient of variation (CV) values were calculated for each sample and both levels of control.

Stability assessment of EDTA samples

Twenty samples with ESR results were randomly selected for the stability study. Ten samples were kept at room temperature (24-26ºC) and the remaining 10 were kept in a refrigerator (4°C). ESR was measured again on the Vision C instrument at the fourth, eighth, and twenty-fourth hour. The samples kept at 4°C were measured when they reached room temperature. The results at 4, 8, and 24 hours were compared with the first (0 hour) results, as in 2 studies performed by Lapic et al. [13, 14].

Method comparison study

A total of 173 EDTA anticoagulated blood samples were included in the method comparison study. Two sequential ESR measurements were completed within 4 hours after blood collection at room temperature (24-26ºC) by a single trained laboratory technician. After the direct measurement of ESR on the Vision C instrument in 20 minutes, the same EDTA anticoagulated sample was used for a manual determination of ESR according to the Westergren method (Sediplast; LP Italiana S.p.A, Milan, Italy). A test tube containing 0.2 mL of sodium citrate was filled with 0.8 mL of EDTA-anticoagulated blood and gently mixed at least 12 times. A scaled pipette 200 mm in length with an internal diameter of 2.5±0.15 mm was carefully pushed over the cap, and constant contact of pipette with the

bottom of the tube was maintained and kept in an upright position. After 60 minutes, the vertical fall of the erythrocytes in the plasma was determined visually and the results were recorded by hand.

Statistical analysis

Microsoft Excel software (Microsoft Corp. Redmond, WA, USA) was used to calculate the mean, SD, and CV for intra-run and inter-run precision. MedCalc Statistical Software version 18.11.6 (MedCalc Software bv, Ostend, Belgium) and IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) were used to perform additional statistical analysis. The Wilcoxon signed rank test was applied to examine stability analysis, a Bland-Altman plot was used for bias assessment, Passing-Bablok regression analysis was performed to evaluate the systematic and proportional error, and nonparametric Spearman rank correlation (rho correlation coefficient) was used for correlation analysis. Statistical significance was accepted at $p<0.05$.

Results

The intra-run precision ranged from 4.93% to 18.18% for 5 samples at different levels. The inter-run CV% for the first level control sample was 7.03% and 2.94% for the second level control sample. The results of intra-run and inter-run precision are shown in Table 1.

The 24-hour stability study revealed no statistically significant difference in the measurement performed at the fourth hour compared with the first (0 hour) measurement, while a statistically significant difference was seen in the measurements performed at 8 and 24 hours in comparison with the first (0 hour) measurement. Table 2 illustrates the results of the stability study.

For method comparison, 173 patient samples were studied using both methods. The ESR results obtained with the Westergren method were divided into 3 groups: <40, 40-80, and >80 mm/hour. The mean age of the 173 patients (94 female/79 male) was 51.12±18.26 years and the mean hematocrit value was 41.42±3.39%. Characteristics of gender and age distribution, hematocrit ratio, and ESR levels are shown in Table 3. A Bland-Altman plot illustrates the bias between the Vision C device and the Westergren method in Figure 1 [15]. In Figure 2, the regression equation resulting from Passing-Bablok regression analysis [16] of the Vision C analyzer and the Westergren method was y=2.073+0.805x. The bias, slope, and intersection values obtained according to 3 ESR levels are provided in Table 4.

Table 1. Intra-run and inter-run precision data of the Vision C analyzer

	Intra-run precision* Samples					Inter-run precision** Bio-Rad control	
	1	2	3	4	5	Level 1	Level 2
Mean (mm/h)	2.2	18.9	30.6	66.7	71.7	3.00	42.79
SD	0.4	1.81	3.35	3.28	5.48	0.21	1.26
CV%	18.18	9.60	10.96	4.93	7.64	7.03	2.94

CV: Coefficient of variation, SD: Standard deviation. *ESR measurement of 5 randomly selected samples was repeated 10 times in 8 hours. **Two level controls (Bio-Rad Liquicheck Sedimentation Rate Control Level 1, Level 2) were studied 3 times a day for 5 consecutive days

Table 2. Evaluation of sample stability results for the Vision C analyzer

	0 hour	4 hours	8 hours	24 hours
25°C				
Mean±SD (mm/h)	32.5±23.91	29.4±22.0	26.5*±21.38	14.1*±10.63
Mean of differences (mm/h)		3.1	6	18.4
95% CI		-0.78-6.98	2.14-9.85	6.49-30.30
p		0.113	**0.013**	**0.008**
4°C				
Mean±SD (mm/h)	31.7±19.91	26.6±21.21	25.6*±21.71	20.1*±21.02
Mean of differences (mm/h)		5.1	6.1	11.6
95% CI		-0.09-10.28	2.69-9.51	8.54-14.66
p		0.052	**0.012**	**0.00**

20 samples were selected randomly: 10 samples were stored at room temperature (25°C) and 10 samples were stored in a refrigerator (4°C). Erythrocyte sedimentation rate measurement was performed using a Vision C analyzer at 4, 8, and 24 hours. *: statistically significant difference compared with the 0-hour measurement

Table 3. Age, gender, and hematocrit level distribution of the groups and erythrocyte sedimentation rate measurements using 2 methods

	<40 mm/hour (n=113)	40-80 mm/hour (n=30)	>80 mm/hour (n=30)
Age (Years)	46.9±18.7	58.1±15.5	59.9±13.7
Gender (Female/male)	64/49	18/12	12/18
Hematocrit (%)	42.5±3.14	40.65±2.87	38.13±2.33
Vision C (Mean±SD)	14.99±10.27	44.80±9.82	76.43±10.26
Westergren (Mean±SD)	15.96±11.23	54.03±12.43	93.7±12.51

Table 4. Comparison of the Vision C analyzer and the Westergren method results

	<40 mm/hour (n=113)	40-80 mm/hour (n=30)	>80mm/hour (n=30)
Bias (CI)	-0.885 (-2.027 to 0.258)	-9.23 (-11.853 to -6.613)	-17.26 (-21.306 to -13.227)
Equation (y)	1.5+0.875x	3.875+0.75x	22.775+0.575x
Intercept (CI)	1.5 (0.0 to 2.42)	3.875 (-8.5 to 12.76)	22.775 (12.3 to 47.4)
Slope (CI)	0.875 (0.79 to 1.0)	0.75 (0.589 to 1.000)	0.575 (0.3 to 0.94)
Correlation coefficient	0.835	0.798	0.526

CI: Confidence interval

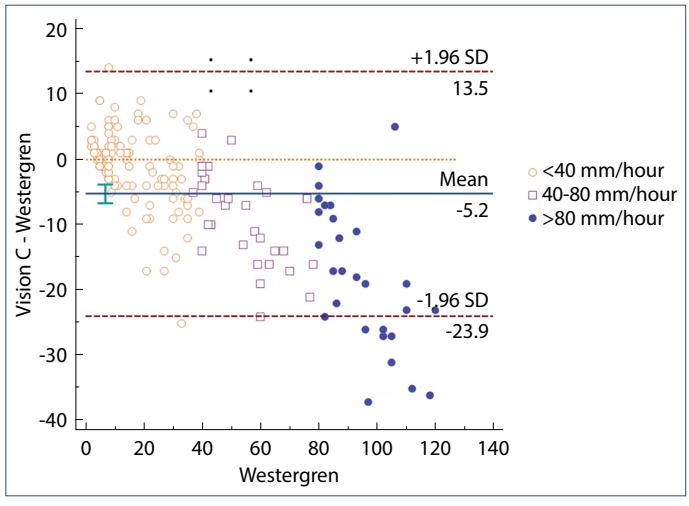

Figure 1. Bland-Altman difference plot of the Westergren method and Vision C analyzer results.

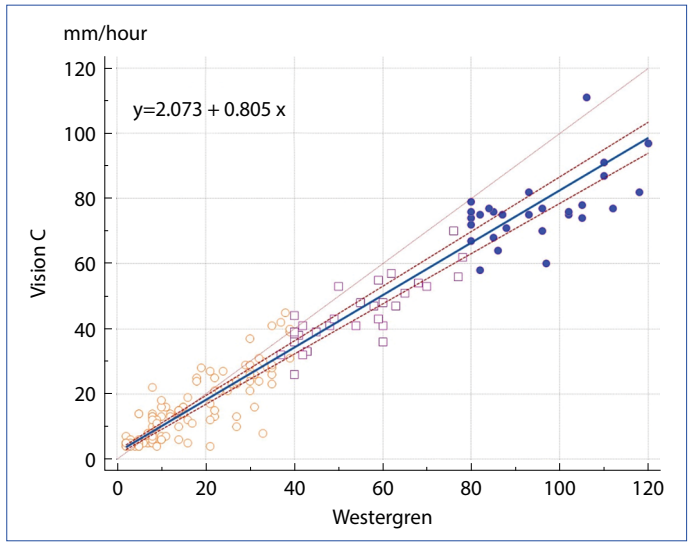

Figure 2. Passing-Bablok regression analysis of the Westergren method and Vision C analyzer results.

Discussion

In recent years, many laboratories around the world have elected to use modified Westergren or alternative ESR methods rather than the reference Westergren method. The new methods yield faster results, require less manpower, automatically perform the sample turning process, and results are easily transferred to the laboratory automation system. The use of a blood collection tube containing EDTA allows for ESR measurement from the same tube that is used to perform whole blood analysis. In addition, some sedimentation instruments can be connected to a whole blood analysis instrument. According to a survey conducted by the ICSH Working Group, only 28% of 6333 laboratories participating in the survey used the standard Westergren method, while 72% used modified or alternative methods. Due to this diversity, the ICSH has defined some validation and verification criteria to standardize instruments that measure ESR using modified or alternative methods [11].

In the current study, we evaluated the analytical performance of the Vision C analyzer by performing precision, stability, and

comparison studies with the reference method. The CV% values obtained from the intra-run precision study of the Vision C device were high (18.18%) at low ESR levels (2.2±0.4 mm/hour), but lower (4.93%) at high ESR levels (66.7±3.28 mm/hour). The same was observed in the evaluation of the precision between days. Similar results have been obtained in studies performed with different instruments and methods [13, 14, 17]. The CLSI H2-A4 guideline states that the acceptable CV% for different ESR levels ranged from 10.8 to 38.88, and that the CV% value was higher at low ESR values, while lower at high levels [18]. In our evaluation, the Vision C instrument returned results with a clinically insignificant level of imprecision at low ESR levels.

In all of the Vision C samples evaluated in terms of stability, a decrease in ESR results was observed over time, regardless of room temperature or refrigerated conditions. Similarly, in a validation study performed with the VesMatic Cube (Diesse Diagnostica Senese S.p.A., Monteriggioni SI, Italy), which uses the modified Westergren method like the Vision C, it was demonstrated that the ESR levels of the waiting samples were lower under all conditions [14]. In another study performed with a VesMatic Cube device, a significant decrease in the ESR value after the sixth hour at room temperature was observed compared with the first measurement, but there was no significant difference in the ESR value of samples kept in the refrigerator [17]. According to our data, there was a statistically significant difference between the ESR measurements at room temperature and the refrigerator at the eighth hour. These findings support the requirement ESR measurement should be conducted within 4 hours at room temperature [10].

For a method comparison study, the ICSH recommends covering the whole 0-120 mm hour range and including at least 20 samples for 3 different ESR levels and that the hematocrit levels of all samples should also be within reference ranges [11]. In the present study, the sample group at the level of <40 mm/hour comprised 113 patients, and the groups at the level of 40-80 mm/hour and >80 mm/hour each included 30 patients. The correlation coefficient, Passing-Bablok regression analysis results, and Bland-Altman plots were used to compare the 2 ESR methods as indicated in CLSI document A02-A5 and ICSH recommendations [10, 11]. The overall bias of the Vision C analyzer was -5.23 mm/hour (confidence interval [CI]: -6.66 to -3.79) and the correlation coefficient of the 2 methods was 0.948. The Passing-Bablok regression equation was (y=2.073+0.805x) with constant (intercept CI: 1.400 to 2.685) and proportional (slope CI: 0.771 to 0.840) error. When the 3 ESR level groups were examined, the bias was -0.885 (CI: -2.027 to 0.258) for values <40 mm/hour, -9.23 (CI: -11.853 to -6.613) for values between 40-80 mm/hour, and -17.26 (CI: -21.306 to -13.227) for values >80 mm/hour. Based on these data, we observed that the Vision C provides an acceptable alternative to the Westergren method at ESR values <40 mm/hour since the bias was not significant and neither a constant nor a proportional error was seen at these low levels. However, at medium ESR levels, the bias increased to a significant level (-9.23) and at high ESR levels, in addition to a high bias (-17.26), a constant and proportional error was obtained as well. In our opinion, this high bias (-17.26) and systematic (constant and proportional) error may pose a problem for high ESR values when used for the diagnosis and prognosis of disease.

We found no study in the literature that compared the Vision C with the reference method Westergren method. In a study comparing the VesMatic CUBE 200, which uses a modified Westergren method, with the reference Westergren method, the authors reported no bias between the 2 methods, a correlation coefficient of 0.852, and a small constant error in the regression curve. It is thought that this constant error may be due to the difference in the reading time between the methods and the extrapolation made by the modified method to convert the 20-minute measurement to 60 minutes [13, 14]. Boğdaycıoğulları et al. [17] also compared the VesMatic CUBE 200 with the Westergren method and reported a bias of 16.7%, and a systematic proportional error at high ESR values, which is similar to our findings. Several studies have suggested careful use of non-standard Westergren methods [11, 17, 19]. While newer methods seem practical and advantageous, they need standardization. In a study that examined the results of external quality control, it was observed that there were differences of up to 142% between the Westergren method and other methods [11].

Interference studies (hemolysis, fibrinogen) were not performed in this research. This is a limitation of our study.

Conclusion

In conclusion, the Vision C instrument, which uses a modified Westergren method and EDTA blood collection tubes, met the intra-run and inter-run precision criteria. However, it was not fully compatible with the Westergren method at all ESR levels and a significant difference was observed at high ESR levels. This may pose a problem, particularly in the follow-up of patients for whom ESR measurement is performed due to chronic disease. To overcome this situation, follow-up of these patients should be carried out with the same laboratory and the same method. Furthermore, it may be advisable to report the ESR measurement method used in laboratory reports and inform clinicians when the method is changed.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

Ethics Committee Approval: Ethics approval for this study was granted by the Suleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee on May 7, 2019 (no: 154).

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References

1. Brigden ML. Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician 1999;60(5):1443–50.
2. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. JAMA 2018;320(13):1360–72. [CrossRef]
3. Dasgupta B, Cimmino MA, Maradit-Kremers H, Schmidt WA, Schirmer M, Salvarani C, et al. Provisional criteria for polymyalgia rheumatica: a European league against rheumatism/American college of rheumatology collaborative initiative. Ann Rheum Dis 2012;71(4):484–92. [CrossRef]
4. Ness T, Bley TA, Schmidt WA, Lamprecht P. The diagnosis and treatment of giant cell arteritis. Dtsch Arztebl Int 2013;110(21):376–86. [CrossRef]
5. Barrack R, Bhimani S, Blevins JL, Blevins K, Demetres M, Figgie M, et al. General assembly, diagnosis, laboratory test: proceedings of international consensus on orthopedic infections. J Arthroplasty 2019;34(2S):S187–95. [CrossRef]
6. Eichenauer DA, Aleman BM, André M, Federico M, Hutchings M, Illidge T, et al. Hodgkin lymphoma: ESMO clinical practice guidelines for diagnosis, treatment, and follow-up. Ann Oncol 2018;29(4):19–29. [CrossRef]
7. Westergren ALF. Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Medica Scandinavica 1921:54(1);247–82. [CrossRef]
8. Reference method for the erythrocyte sedimentation rate (ESR) test on human blood. Br J Haematol 1973;24(5):671–3.
9. Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S; International Council for Standardization in Haematology. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol 2011;33(2):125–32. [CrossRef]
10. Clinical and Laboratory Standards Institute (CLSI). Procedures for the erythrocyte sedimentation rate test; approved standard-fifth edition. CLSI document H02-A5. Wayne, Pennslyvania: Clinical and Laboratory Standards Institute; 2011.
11. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ. International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hematol 2017;39(5):448–57. [CrossRef]
12. Manley RW. The effect of room temperature on erythrocyte sedimentation rate and its correction. J Clin Pathol 1957;10(4):354–6.
13. Lapic I, Piva E, Spolaore F, Musso G, Tosato F, Pelloso M, et al. Ves-Matic CUBE 200: is modified Westergren method for erythrocyte sedimentation rate a valid alternative to the gold standard? J Clin Pathol 2019;72(10):716–9. [CrossRef]
14. Lapić I, Piva E, Spolaore F, Tosato F, Pelloso M, Plebani M. Automated measurement of the erythrocyte sedimentation rate: method validation and comparison. Clin Chem Lab Med 2019;57(9):1364–73. [CrossRef]
15. Giavarina. D. Understanding Bland Altman analysis. Biochemia Medica 2015;25(2):141–51. [CrossRef]
16. Bilić-Zulle L. Comparison of methods: passing and bablok regression. Biochem Med (Zagreb) 2011;21(1):49–52. [CrossRef]
17. Boğdaycioğlu N, Yilmaz FM, Sezer S, Oğuz E. Comparison of iSED and ves-matic cube 200 erythrocyte sedimentation rate measurements with westergren method. J Clin Lab Anal 2015;29(5):397–404. [CrossRef]
18. NCCLS. Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition. NCCLS document H2-A4. Pennsylvania, USA: NCCLS; 2000.
19. Schapkaitz. E, Rabu Rabu S, Engelbrecht M. Differences in erythrocyte sedimentation rates using a modified Westergren method and an alternate method. J Clin Lab Anal 2019;33(2):e22661.