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Journal of Hematology and Allied Sciences


*Original Article*

# Comparison of alternate erythrocyte sedimentation rate measurement by automated Celltac α+ (MEK 1305) and reference Westergren method

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Received : 11 April 2022
Accepted : 26 June 2022
Published : 21 October 2022

**DOI**
10.25259/JHAS_12_2022

**Quick Response Code:**

## ABSTRACT

**Objectives:** Manual Westergren method is routinely used for ESR measurement; however, it has many limiting factors which include inherent and technical factors. Alternate/modified Westergren methods have been devised to overcome the limitations of the manual Westergren method. These new methods must be properly evaluated before introducing in clinical laboratories.

**Material and Methods:** A total of 350 randomly collected Ethylene Diamine Tetraacetic Acid (EDTA) samples from hospitalized and ambulatory patients were assayed parallelly in the recently launched Hematology Analyzer Celltac α+ (MEK 1305) and manual Westergren method. Results of these assays were subjected to statistical analysis using a coefficient of correlation, Passing-Bablok regression, and the Bland-Altman statistical methods and the results of the present study were also compared with 16 selected similar studies published in the past 22 years. Intrarun precision using patient samples and inter-run precision using manufacturer's controls (MEK-3DN and MEK-3DL) were also determined.

**Results:** The present study revealed a Pearson correlation of 0.9058, mean bias of −6.43, and limits of agreements 17.5−−30.4, between Celltac α+ (MEK 1305) and the reference Westergren method. Intrarun precision using patient samples yielded a coefficient of variation ranging from 0% to 14% and inter-run precision using commercial controls yielded a CV of 9.32% for abnormal (MEK-3DL) control and 13.6% for normal level control (MEK-3DN).

**Conclusion:** With good correlation between Celltac α+ (MEK 1305) and the reference Westergren method, acceptable bias, and limits of agreements, automated Celltac α+ (MEK 1305) with its additional advantages is a valid substitute for the reference ESR method in clinical laboratories.

**Keywords:** Celltac α+, ESR, Comparison, Passing-Bablok, Syllectogram

## INTRODUCTION

The erythrocyte sedimentation rate (ESR) is widely used laboratory test in clinical laboratories. ESR is the sedimentation of the red cell by observing the level to which the cells fall in a given time interval, usually 1 h in a well-described specific pipette.[1]

ESR is an informative inflammatory condition, including rheumatoid arthritis, giant cell arthritis, polymyalgia rheumatica, and other connective tissue disorders.[2]

However, ESR measurement by the Westergren method has several limitations such as long analytical time, requirement for large specimen volume, need for diluting specimens, biohazards,

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and elevation of ESR by anemia. Technical factors such as variation at room temperature, time from specimen collection to test setup, tilting and vibrations, exposure to direct sunlight, improper filling, inconsistent internal boreholes of Westergren tubes, inaccuracy in reading meniscus lines in hazy samples, affect ESR test results, and necessitating the use of alternate test methods.

Alternate ESR methods (instruments not based on the Westergren method) use novel approaches such as centrifugation or photometric rheology.[3,4]

Celltac α+ (MEK 1305) is an automated hematology analyzer for complete blood counts (CBCs) with an added facility for ESR estimation. This analyzer uses an optical measurement of the Rouleaux formation and aggregation of RBCs that occur in the initial phase of the sedimentation phenomena to measure ESR in a short amount of time.[5]

The present study aimed to evaluate the analytic performance of Celltac α+ (MEK 1305) for ESR measurement and compare it with the Westergren reference method. As per the recommendation of the International Council for Standardization in Hematology (ICSH), Passing-Bablok regression and the Bland-Altman method were used as the main statistical tools in the present study.[6]

## MATERIAL AND METHODS

### Samples

The study was conducted at Bhanumati Clinical Laboratory (Navsari, India) using 350 random samples (Confidence level 90%, Margin of error 5%)[7] collected from hospitalized and ambulatory patients from September 2021 to October 2021.

Hemolyzed and clotted samples were excluded. A 3 ml of blood was collected in Labtech Disposable blood collection tubes (product by Labtech Disposables, Gandhinagar, India) having K3 EDTA as the anticoagulant. Samples were stored at room temperature and analyzed within 4 h of collection, both by manual Westergren method and by Celltac α+ (MEK 1305) analyzer.

### ESR measurement by Celltac α+ (MEK 1305)

Automated mixing of EDTA sample tube was performed for 2 min to ensure complete disaggregation of erythrocyte before analyzing the sample in the automated analyzer. Samples were assayed as per the operator's manual for Celltac α+ (MEK 1305).[5]

### Manual measurement of ESR by Westergren method

The same blood EDTA tube used for ESR measurement by Celltac α+ (MEK 1305) was once again used for determining ESR by the Westergren method. Automated mixing of EDTA sample tube was performed for 2 min before setting up the ESR test by Westergren method.

K3-EDTA anticoagulant venous blood diluted 4:1 in citrate was transferred to Hemopette (vacuumized disposable tube). Hemopet (manufactured by Lab Consumables, India) is an ESR pipette, 230 mm in length with an internal bore of 2.55 mm for ESR measurement. During sedimentation, the pipettes were mounted vertically and kept at room temperature.

The distance that the column of blood fell in ESR pipette in one hour was recorded and reported in mm/hour.

The study was conducted in concordance with the principles of Helsinki. Samples were collected anonymously and no identifying information was recorded for the study.

### Quality check of Celltac α+ (MEK 1305) using control materials

Hematology controls, MEK-3DL (Lot No. B218L) and MEK-3DN (Lot No. B218N), from Nihon Kohden Corporation, Tokyo, Japan, were assayed once a day at the start of the shift to ensure the appropriate functioning of the Celltac α+ (MEK 1305) analyzer.

### Precision check of Celltac α+ (MEK 1305) analyzer using hematology controls and patient samples

Between-run precision was performed with normal (MEK-3DN) and abnormal (MEK-3DL) controls analyzed once a day for 40 consecutive days. Results of 40 consecutive assays of MEK 3DL and MEK 3DN controls were used to determine precision for the Celltac α+ (MEK 1305). Moreover, data of nine random patient samples (each assayed 11 times on same day) on the hematology analyzer were also used to determine precision (repeatability).

### Statistical analysis

For method comparison study, EDTA blood samples were analyzed in parallel with Westergren method and MEK 1305 analyzer. For the evaluation of the MEK 1305 analyzer, coefficient of correlation, Passing-Bablok regression, and Bland-Altman statistical methods were used.[6]

Bias, accuracy, and limits of agreement were derived using the Bland-Altman plot.

The calculations were performed using "MedCalc" statistical software (version 20) and the "Statistical Package for the Social Sciences" (version 26).

## RESULTS

A total of 350 random samples were assayed parallelly by the Westergren method and Celltac α+ (MEK 1305) and showed results as follows.

ESR values measured with the Westergren method (mean 31.02 ± SD 26.6, range 1–142 mm/h) were not significantly different from Celltac α+ (MEK 1305) (mean 24.5 ± SD 28.7, range 0–161 mm/h). The 350 ESR results were segregated into three different groups: 1–20 mm/h, 21–60 mm/h, and more than 60 mm/h.[6]

The results for ESR measured for 350 samples by Westergren and Celltac α+ (MEK 1305) in different ESR ranges along with the statistical calculations (mean, standard deviation, and standard error of mean) are shown in [Table 1].

### Method comparison results

Measurement of ESR in 350 samples resulted in a median of 22 mm with Westergren method and 15 mm with Celltac α+ (MEK 1305). The obtained spearman rank correlation coefficient ρ was 0.909 (95% confidence interval [CI] 0.889–0.926, $P < 0.0001$).

The calculated mean of differences between the two methods (bias) was −6.43 mm/h, where zero is the predicted value if the two methods are identical in their measurements for the same sample. The limits of agreement between the two methods were 17.49 and −30.35.

Method comparison results (Spearman rank correlation coefficient ρ, intercept, slope, and mean bias) between ESR measurement by Westergren and Celltac α+ (MEK 1305) when evaluated separately in three groups as per recommendation of ICSH, 1–20 mm/h, 21–60 mm/h, and >60 mm/h and also for all the results are shown in [Table 2].

Figures 1 and 2 the results of Passing-Bablok regression analysis [Figure 1] and displays the Bland-Altman plot distribution of difference around fitted regression [Figure 2].

### Results for precision studies using commercial control and patient samples

#### *Inter-run precision*

Analysis of commercial control samples and patient samples once a day for 40 days yielded inter-run CV of 9.32% for the abnormal range control (MEK 3DL) and 13.6% for the normal range control (MEK 3DN).

#### *Intrarun precision*

Nine patient samples were assayed 11 times for assessing intrarun precision. Intrarun CV% obtained ranged from 0% to 14%.

## DISCUSSION

ESR is one of the most common worldwide used laboratory tests. It is inexpensive and easy to perform in laboratories of various sizes. It reflects both *plasma properties,* that is, the concentration of acute-phase proteins and *cellular properties*, that is, red cell concentration and aggregation behavior.[8]

**Table 1:** Results obtained by in the present study by Westergren method and Celltac α+ (MEK 1305) for different ESR ranges with their calculated mean, standard deviation, and standard error of mean.

| ESR range | Total samples | Method | Mean (mm/h) | Standard deviation (mm/h) | Standard error of mean |
|---|---|---|---|---|---|
| 1–20 mm/h | 161 | Westergren | 11.1 | 5.3 | 0.4 |
| | | MEK 1305 | 5.6 | 6.0 | 0.4 |
| 21–60 mm/h | 140 | Westergren | 34.9 | 11.0 | 0.9 |
| | | MEK 1305 | 27.8 | 16.4 | 1.3 |
| >60 mm/h | 49 | Westergren | 85.1 | 18.2 | 2.6 |
| | | MEK 1305 | 77.7 | 31.4 | 4.4 |
| Complete range (1–161) mm/h | 350 | Westergren | 31.0 | 26.6 | 1.4 |
| | | MEK 1305 | 24.5 | 28.7 | 1.5 |

**Table 2:** Method comparison results between ESR measurement by Westergren and Celltac α+ (MEK 1305).

| | *n* | ρ (95% confidence interval) | Intercept (95% confidence interval) | Slope (95% confidence interval) | Mean bias (95% confidence interval) |
|---|---|---|---|---|---|
| Low range (1–20 mm) | 161 | 0.678 (0.585–0.754) | −4.00 (−6.00–−3.00) | 0.80 (0.66–1.00) | −5.55 (−6.38–−4.71) |
| Middle range (21–60 mm) | 140 | 0.654 (0.547–0.739) | −21.75 (−31.75–−13.75) | 1.41 (1.16–1.75) | −7.12 (−9.24–−4.99) |
| Upper range (>60 mm) | 49 | 0.644 (0.443–0.783) | −67.7 (−113.0–−41.42) | 1.70 (1.39–2.25) | −7.34 (−13.86–−0.83) |
| All ranges | 350 | 0.909 (0.889–0.926) | −6.16 (−7.0–−5.0) | 0.96 (0.90–1.00) | −6.43 (−7.71–−5.14) |

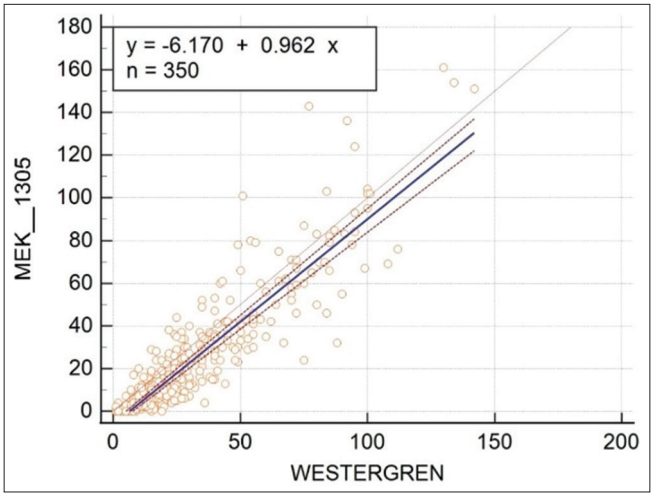


**Figure 1:** Passing-Bablok analysis for comparison of Celltac alptha+ (MEK 1305) and the Westergren method; y = −6.170 + 0.962x.

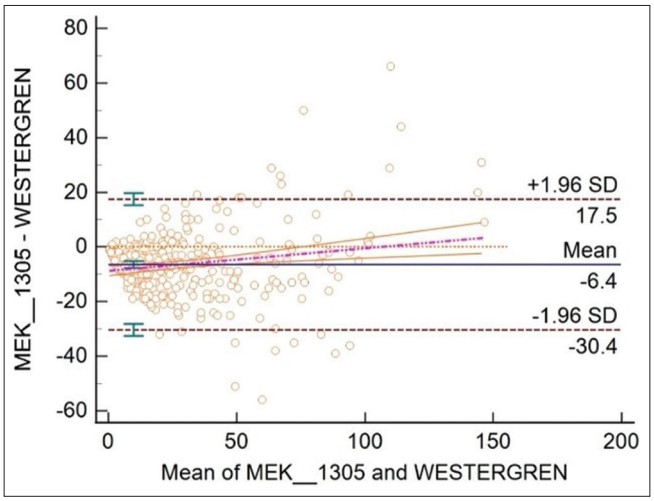


**Figure 2:** Bland-Altman plot of the difference between ESR values obtained with Westergren method and those given by the Celltac α+ (MEK 1305) (Y-axis) versus mean of the ESR values (Westergren + Celltac α+ MEK 1305) (X-axis). Dotted lines denote limits of agreement (−30.4 to 17.5 mm/h), bias is –6.4.

Size, shape, and number of red blood cells, fibrinogen concentration, globulin concentration, and temperature are some of the parameters determining ESR.[9]

Many internal and external factors can influence the find ESR results obtained by the Westergren method. Moreover, the modified Westergren method is laborious, cumbersome, needs large volume of blood, long analysis time (>1 h), and carries the risk of infection.

For practical reasons, the Westergren method is diminishingly used for ESR determination and majority of laboratories have started using alternate or modified methods.

Celltac α+ (MEK 1305) uses optical measurement of the Rouleaux formation and aggregation of red blood cells that occur in the initial phase of the sedimentation phenomenon to measure ESR in a short amount of time. In fact, Rouleaux formation and aggregation of red blood cells are not precisely equivalent to the actual sedimentation rate, but by utilizing the hematocrit (HCT) and mean cell volume (MCV) measurement results, which are closely related to ESR (1 h value), the analyzer can obtain in a short amount of time an ESR value that has a high correlation with the reference method.[5]

The sample volume needed for the CBC plus ESR determination by MEK 1305 analyzer is 80 microliter and the analyzer has a throughput of 20 samples per hour for CBC + ESR mode, giving assay time of 3 min per sample.[5]

**Measurement method for ESR in Celltac α+ (MEK 1305)[5]**

In the ESR measuring unit, light from an LED is emitted onto the agitated blood, and the light that passes through is continuously measured by a light-receiving element. The Rouleaux formation and aggregation of the red blood cells begin as soon as agitation end, causing the intensity of the light passing through the blood to change over time. The waveform expressing this change in light transmission over time is called a syllectogram.

At the same time, the CBC measuring unit measures HCT and MCV. The automated hematology analyzer uses a calculation method to calculate ESR (1 h value), based on the HCT and MCV values obtained by the CBC measuring unit, and the syllectogram produced by the ESR measuring unit.

In the present study, 350 EDTA samples randomly collected from outpatients and inpatients irrespective of age and sex were assayed parallelly by the Westergren method and by Celltac α+ (MEK 1305) hematology analyzer. The Pearson correlation coefficient r obtained in the study was 0.905 indicating a good correlation between Westergren and Celltac α+ (MEK 1305).

Moreover, linear regression analysis according to Passing-Bablok (Y = −6.169811 + 0.962264 x) indicated high concordance between results obtained by study analyzer and the Westergren analyzer.

Bland-Altman analysis demonstrated an acceptable overall bias of −6.4 and limits of agreement of −30.0–17.4 for 350 samples. The bias in the study was increasing for higher values of ESR; bias of −5.5 in low range (1–20 mm), bias of −7.12 in the middle range (21–60 mm), and bias of −7.34 (>60 mm).

The above method comparison obtained in the present study is comparable with selected 16 similar published studies evaluating performance of various alternate/modified Westergren methods for ESR determination against reference Westergren method [Table 3].[10-23]

**Table 3:** Comparative data of selected 16 studies on performance evaluation of different alternate/modified Westergren methods for ESR determination against reference Westergren method.

| S. No. | Author | Year | Equipment used | Sample size | Linear regression | Passing-Bablok | Bland-Altman | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Bias (mm/h) | Limits of agreement | 95% CI |
| 1. | Plebani *et al.*[10] | 1998 | Test 1 | 297 | r=0.85<br>Y=0.99x+2.39<br>*P*=0.0001 | - | −1.39 | −40.72–37.94 | −3.77–−0.99 |
| 2. | Romero *et al.*[11] | 2003 | Test 1 | 131 | ρ=0.917<br>*P*<0.01 | - | 0.99 | −19.88–21.77 mm | −0.81–2.78 |
| 3. | AlFadhli and Al-Awadhi[12] | 2005 | SEDI system | 150 | r=0.91<br>*P*<0.0001 | - | −13.18 | −37.88–11.52 | - |
| 4. | Ozdem *et al.*[13] | 2006 | Test 1 | 113 | r=0.94<br>*P*<0.0001 | - | −0.29 | −14.5–13.9 | −1.06–1.65 |
| | | | SRS 100 | | r=0.94<br>*P*<0.0001 | - | 1.63 | −15.8–19.1 | −3.29–0.03 |
| 5. | Mahlangu and Davids[14] | 2008 | HumaSed ESR Auto Plus | 125 | r=0.93<br>r=0.96 | -<br>- | 0.60<br>6.60 | −20.2–21.3<br>−10.7–23.8 | −3.7–1.2<br>5.0–8.1 |
| 6. | Cha *et al.*[15] | 2009 | Test 1 | 154 | $r^2$=0.386<br>*P*<0.0005<br>y=0.547x+14.881 | - | −10.95 | −29.9–51.8 | −14.27–−7.63 |
| 7. | Perovic *et al.*[16] | 2010 | Ves-Matic Cube 200 | 250 | ρ=0.946<br>*P*<0.001<br>y=−0.0435+1.0435 | - | −0.5 | −13.0–12.9 | −0.37–1.32 |
| 8. | Hardeman *et al.*[8] | 2010 | Test-1 | 680 | R=0.8996<br>*P*<0.0001<br>y=0.7727+0.9697x | - | 2.0 | −16.4–20.3 | - |
| 9. | Curvers *et al.*[9] | 2010 | SEDI system | 92 | r=0.96<br>y=0.91x+0.77 | - | −1.0 | −22.3–20.3 | −3.3–1.2 |
| | | | Starrsed | 50 | r=0.96<br>y=1.22x+3.14 | - | 10.8 | −9.3–30.9 | 7.9–13.7 |
| | | | Ves-Matic Cube 200 | 119 | r=0.83<br>y=0.99x−2.32 | - | −5.7 | −50.8–39.4 | −9.9–−1.6 |
| 10. | Horsti *et al.*[17] | 2010 | Starrsed | 200 | - | $R^2$=0.72<br>*P*<0.01<br>y=(1.066x−0.24) | 4.1 | - | - |
| 11. | Sezer *et al.*[18] | 2013 | Ves-Matic Cube 200 | 101 | ρ=0.82<br>*P*=0.000 | y=1.15x−2.59 | −0.7 | −32.6–31.2 | - |
| 12. | Boğdaycioğlu *et al.*[19] | 2014 | iSED | 136 | r=0.76<br>*P*<0.0001 | y=0.74x+0.07 | 13 | −35.7–61.6 | - |
| | | | Ves-Matic Cube 200 | | r=0.84<br>*P*<0.0001 | y=0.92x+1.25 | 1.4 | −34.4–37.2 | - |
| 13. | Schapkaitz *et al.*[20] | 2018 | iSED | 120 | r=0.88<br>*P*<0.001 | - | 7.99 | - | −5.87–10.13 |
| 14. | Kim *et al.*[21] | 2018 | Test 1 | 195 | | y=0.7323x−8.221 | −26.7 | −84.0–30.7 | −30.81–−22.55 |
| 15. | Lapić *et al.*[22] | 2019 | Ves-Matic Cube 200 | 448 | ρ=0.852<br>*P*<0.001 | y=0.98x+1.4 | −0.3 | −33.7–33 | −1.9–1.2 |
| 16. | Maki *et al.*, 2021[23] | 2021 | Celltac α+ | 271 | r=0.945<br>*P*<0.001 | y=1.026x+0.46 | - | - | - |
| 17. | Current Study | 2021 | Celltac α+ MEK 1305 | 350 | r=0.9058<br>*P*<0.0001 | y=0.962x−6.169 | −6.43 | −30.4–17.5 | −7.71–−5.14 |

Intrarun precision results obtained by analyzing nine patient samples in 11 replicates yielded coefficient of variation (CV) % ranging to 0–14% which is comparable with similar studies [Table 4].

Inter-run precision results obtained by analyzing commercial controls for low (MEK 3DL) and normal (MEK 3DN), analyzed once a day for 40 consecutive days, respectively, CV% of 9.32% and 13.6% are comparable with studies

**Table 4:** Comparison of intrarun precision results of various studies and present studies using patient samples.

| S. No. | Study and year of publication | Instrument | Number of used study | Number of replicates | ESR range mm/h | Coefficient of variation % |
|---|---|---|---|---|---|---|
| 1. | Plebani *et al*., 1998[10] | Test 1 | 7 | 10 | 10–117 | 2.8–7.5 |
| 2. | Romero *et al*., 2003[11] | Test 1 | 2 | 10 | - | 3.37–4.86 |
| 3. | Ozdem *et al*., 2006[13] | Test 1 | - | 10 | 5–61 | 1.4–7.2 |
| | | SRS 100 | - | 10 | 5–65 | 2.8–11.8 |
| 4. | Perovic *et al*., 2010[16] | Ves-Matic Cube 200 | - | 10 | 9–95 | 5.66–13.88 |
| 5. | Horsti *et al*., 2010[17] | Starrsed | - | 6 | - | 0 |
| 6. | Sezer *et al*., 2013[18] | Ves-Matic Cube 200 | - | 20 | 3–106 | 5.69–14.99 |
| 7. | Boğdaycioğlu *et al*., 2014[19] | iSED | - | 20 | 13.7–95.4 | 8.8–14.21 |
| | | Ves-Matic Cube 200 | - | 20 | 3.6–90.3 | 9.9–19.99 |
| 8. | Schapkaitz *et al*., 2018[20] | iSED | - | - | 13.5–85 | 8.7–15.3 |
| 9. | Lapić *et al*., 2019[22] | Ves-Matic Cube 200 | 11 | 5 | 4.6–107.8 | 2.2–10. 7 |
| 10. | Maki *et al*., 2021[23] | Celltac α+ | 12 | 10 | 1–157 | 3.3–15.1 |
| 11. | Present study | Celltac α+ MEK 1305 | 9 | 11 | 1–38.3 | 0–14 |

published by Mahlangu and Davids,[14] Horsti *et al*.,[17] and Schapkaitz *et al*.[20]

Celltac α+ (MEK 1305) being an automated closed system offers many advantages such as ease of performance, safety, savings on consumables, use of EDTA sample, need for less sample volume, and less turnaround time. The analyzer additionally gives the results in the CBC + ESR mode adding value to its utility in the hematology laboratories.

With Pearson correlation r of 0.9058, mean bias of −6.43 across the range of ESR and limits of agreement 17.5 to −30.4, between Celltac α+ (MEK 1305) and reference Westergren method, Celltac α+ (MEK 1305) is reliable automated analyzer to determine ESR in clinical laboratories.

## CONCLUSION

Celltac α+ (MEK 1305) analyzer showed good correlation with the conventional Westergren method and an acceptable bias over the entire range of ESR, exhibiting satisfactory concordance of ESR results between Celltac α+ (MEK 1305) and reference Westergren method. Celltac α+ (MEK 1305) analyzer offers major advantages such as use of EDTA sample, reduced sample volume, ease of performance, reduction in biohazard risk, and reliability, making it a valid substitute for reference Westergren method for ESR determination.

### Acknowledgment

The author gratefully acknowledges the help extended by Mr. Nitin Nayyar product specialist and Mr. Rahul Chaudhary National Manager, Nihon Kohden, India, for supplying the Celltac α+ (MEK 1305) analyzer and consumables for this evaluation. The author also thanks Miss. Rikita Vaghasiya and Miss. Neha Patel for processing the samples and typing the manuscript.

### Declaration of patient consent

Patients' consent not required as patients' identity is not disclosed or compromised.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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**How to cite this article:** Kahar MA. Comparison of alternate erythrocyte sedimentation rate measurement by automated Celltac α+ (MEK 1305) and reference Westergren method. J Hematol Allied Sci 2022;2:39-45.