Performance evaluation of alternate ESR measurement method using BC-780 automated hematology analyzer: a comparison study with the Westergren reference method

Abstract

Objectives: Implementation of alternate erythrocyte sedimentation rate (ESR) measurement method is increasing worldwide due to its various advantages. In this study, we aim to evaluate the analytical performance of the BC-780 automated hematology analyzer in measurement of ESR value.

Methods: Analyzer performance including precision study, carryover, sample stability and potential interferences are examined. Samples with ESR values spanning the whole analytical ESR range are included for method comparison study. Samples with different hematocrit (Hct) and mean corpuscular volume (MCV) values are also analyzed and compared with the results obtained from the Westergren reference method.

Results: Precisions and carryover results are consistent with the manufacturers’ claim. ESR values do not change significantly in the samples stored at 2–8 °C for 24 h (h) or at room temperature (RT) for 8 h, but significantly decreased (p<0.001) when stored at RT for 24 h. Significant increase in ESR value is documented in samples that are hemolyzed (hemoglobin concentration ranged from 1.28–6.01 g/L) (p=0.010) or lipemic (triglyceride above 4.75 mmol/L) (p=0.001). Method comparison study yields a proportional difference with a regression equation=3.08+0.98x. Bland–Altman analysis shows a mean absolute bias of 3.12 mm. The obtained absolute mean biases are below 5 mm in all analytical categories except for the group where MCV>100 fL.

Conclusions: Most tested parameters met the manufacturer’s specifications and were comparable to the reference method. Despite the presence of positive bias, it falls within acceptable criteria. Extensive validation against potential interferences such as hemolysis/lipemia is still necessary in future.

Keywords: automation; BC-780; erythrocyte sedimentation rate; Westergren method

Introduction

Although erythrocyte sedimentation rate (ESR) is a non-specific parameter for any specific disease, an elevated ESR value is regarded as a useful indicator for various inflammatory diseases, infections, and malignancies. ESR serves as a valuable diagnostic marker and aids in monitoring treatment progress for conditions such as rheumatoid arthritis [1], polymyalgia rheumatic disease [2], giant cell arteritis [2], orthopedic infection [3], multiple myeloma [4], and Hodgkin’s lymphoma [5]. Consequently, ESR analysis remains widely requested as one of the most common routine laboratory tests, particularly for blood specimens received in tertiary hospitals.

Theoretically, ESR involves three phases for the sedimentation of red blood cells including, (1) formation of erythrocytes stacks called rouleaux formation, which is mostly influenced by the change in plasma protein composition, (2) decantation, and (3) final sedimentation/packing [6]. The Westergren method, recognized by the International Council for Standardization in Hematology (ICSH) as the “gold standard” due to its reproducibility, reliability, and high sensitivity, requires an analysis time of 1 h (h) to complete the three phases [7]. However, this method has several drawbacks, including long analysis time, is considered a biohazard risk as it must be manually tested in an open system using a Westergren tube, and requires high blood volume with a dedicated tube containing a specific anticoagulant (diluted 4:1 in
trisodium citrate), leading to an additional blood draw specifically for ESR measurement only.

Over the past two decades, various modified Westergren methods and alternative techniques have been introduced for measuring ESR. While the modified Westergren method still involves sedimentation measurement, it has been adapted to utilize a shorter reading time of approximately 20–30 min. It has been modified to use primary ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood tubes or to use a lower volume of blood and employ infrared beams to detect the rate of sedimentation [8, 9]. In contrast, alternate methods use different measurement techniques such as photometric rheology [10], capillary photometric-kinetic technology [11] or centrifugation [12]. Most alternate methods use blood from EDTA tubes and are performed in a closed system and measures only the rouleaux formation phase. Mathematical equations are then used to extrapolate the result to 1 h of the Westergren sedimentation. Both modified Westergren and alternate methods are now widely implemented as they provide rapid results, reduce biohazard exposure, use less specimen volume, and use the same type of tubes as the routine CBC test. Most are assembled as automated or semi-automated machines that can reduce the errors of manual testing and reporting. According to the ICSH global survey in 2019, only 28% of laboratories still use the Westergren method, while 72% use modified Westergren or alternate methods. However, the results obtained from non-Westergren instruments might yield a large difference (up to 142%) compared to results obtained by the Westergren method. Thus, the ICSH recommends that both modified Westergren and alternate methods must be evaluated against the Westergren reference method before implementation in clinical laboratories [7].

To follow the ICSH recommendations, the aim of this study is to evaluate the ESR values obtained by the BC-780 automated analyzer which integrates measurement of ESR with routine complete blood count (CBC). The results were then compared with the values measured by the Westergren reference method. Samples with normal CBC results as well as the samples with different ranges of hematocrit (Hct) and mean corpuscular volume (MCV) were included for analysis. Additionally, precision, sample stability, carryover, and potential interfering factors were also explored such as hemolysis, lipemia, and increased fibrinogen concentration.

Materials and methods

Specimen collection

The study was conducted at the Central Laboratory, King Chulalongkorn Memorial Hospital using leftover specimens submitted for routine complete blood count from both in- and out-patient departments. The samples were randomly selected and contained a minimum volume of 2 ml collected in K2-EDTA vacutainers (Becton-Dickinson, NJ, USA). All samples were analyzed within 4 h from venipuncture according to the ICSH recommendations [13] except for the stability study. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Chulalongkorn University (approval number 1689/2022).

The Westergren method

According to ICSH recommendations [7], the Westergren method was used as the reference method as described in our previous study [9]. In detail, a 4:1 ratio of EDTA-anticoagulated blood and 3.8% trisodium citrate dihydrate was mixed and then filled into the Westergren tubes. Samples were placed vertically in the tube holder at room temperature in areas free from vibration. Erythrocyte sedimentation was visually read and recorded as an absolute number after 1 h.

Automated ESR analyzer

ESR value was analyzed by BC-780 automated hematology analyzer (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) using CBC + differential counts (DIFF) + ESR mode. The analyzer measures ESR value using near-infrared photometry to determine the degree of red blood cell aggregation that occurs in the first phase (rouleaux formation) of sedimentation. Erythrocytes are initially disaggregated by high blood flow velocity before abruptly coming to a halt. During this period, reaggregation of erythrocytes is started again. Data on the extent and rate of erythrocyte aggregation are collected within a span of approximately 1.5 min. Subsequently, a mathematical model is applied to calculate the 1-h ESR result, as reported by the Westergren method. For a single test, approximately 160 μL of EDTA blood sample is required for simultaneous measurement of both CBC and ESR [14]. ESR results obtained from the BC-780 automated analyzer were reported as a two-digit value.

Precision study

The precision study protocol was applied according to the procedure documented in the Clinical Laboratory Standards Institute (CLSI) EP15-A3 [15]. Inter-run study was performed using stabilized whole blood quality control (QC) materials (BC-6D, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), including level 1 (MAB112AL, lot number: 27881), level 2 (MAB112AN, lot number: 27882), and level 3 (MAB112AL, lot number: 27882). QC materials were analyzed 3 times per day for 5 consecutive days. Six patient samples with the ESR values spanning the whole analytical range, low (<40 mm), medium (40–80 mm), and high (>80 mm); 2 samples for each range were randomly selected for intra-run precision study. The samples were analyzed 10 times during a 4 h period. Mean, standard deviation (SD), and coefficient of variation (CV) values were calculated for all QC materials (inter-run) and patient samples (intra-run).

Effect of hemolysis, lipemic, and fibrinogen interferences

Hemolysis interference was examined in 14 patient samples covering the whole analytical range using the previously described protocol [9].
Two sets of samples, with and without hemolysis were prepared. In brief, red cell hemolysate was prepared by adding 10 μL of packed red cells into 1,490 μL of ddH2O. To create an artificial hemolysis sample, 100 μL of hemolysate was added to 900 μL of whole blood. Hemoglobin concentration in plasma of the spiked sample ranged from 1.28–6.01 g/L (analyzed by Alinity c System, Abbott Diagnostics, Abbott Park, IL, USA). The same procedure was performed for non-hemolysis samples, but normal saline solution (NSS) was used instead of hemolysate to avoid dilution effect [16]. Lipemic interference (performed in 15 patient samples) was prepared by spiking lipid solution (Intralipid™, Fresenius Kabi, China) which contain 4.73 mmol/L triglyceride (analyzed by Alinity c System, Abbott Diagnostics, Abbott Park, IL, USA) into whole blood at a ratio of 1:100. Similarly, the same volume of NSS was added for a non-lipemic sample. The effect of hemolysis and lipemic on ESR results were calculated as %bias following the previous report [17]. Fibrinogen interference study was conducted by using 4 healthy donors blood samples; 2 male and 2 female. Concentrated fibrinogen solution (20 g/L) was prepared as previously described [7, 9, 17]. Different ratios of NSS and fibrinogen stock (NSS:fibrinogen stock) were prepared including 3:1, 1:1, and 1:3 ratios for a 400 μL volume. Four-hundred microliters of fibrinogen stock solution and NSS were used as negative and positive control, respectively. These procedures yielded working stocks with fibrinogen concentrations of 0, 5, 10, 15, and 20 g/L. Two mL of blood was added into each NSS-fibrinogen mixture tube. For fibrinogen interference study, the prepared mixtures were examined using both automated analyzers as well as the Westergren method.

**Carryover study**

Samples with ESR value of <15 mm/h and >80 mm/h were selected as low target value (LTV) and high target value (HTV), respectively. Three consecutive HTV runs were performed followed by 3 consecutive runs of LTV samples resulting in a total of 6 values named H1, H2, H3, L1, L2, and L3. Three sets of LTV and HTV samples were then analyzed. Carryover was calculated according to the following formula: %carryover = \((L_1 - L_3)/(H_3 - L_3)\) × 100 % [18].

**Sample stability study**

Sample stability was divided into 3 sets of experiments which was only measured by BC-780 automated hematology analyzer. For the first set, stability at RT was performed using 92 randomly selected samples. ESR was first measured on fresh samples (<4 h from venipuncture). Samples were reanalyzed after they were kept at RT for 8 and 24 h after venipuncture. The second set was performed in 138 samples freshly measured and kept at 2–8 °C. The third set was performed in 66 samples that were freshly analyzed and reanalyzed after being kept at RT for 8 h. Refrigerated samples (2–8 °C) from the second and third sets of experiments that were stored up to 24 h from venipuncture were returned to RT prior to re-analysis. A paired samples t-test was used to investigate the differences in ESR values after different storage times and temperatures.

**Method comparison study**

In the method comparison study, ESR values were tested within 4 h from venipuncture. According to ICSH recommendations, only samples with normal CBC values were initially selected for comparison [7]. The ESR values that were measured by the Westergren method span the whole analytical range with at least 20 samples of low (<40 mm/h), medium (40–80 mm/h) and high (>80 mm/h) ranges that were then compared with values measured by the BC-780 analyzer. As Hct level and erythrocyte size or shape could affect the ESR results [7, 19, 20], samples with different Hct and MCV values were also included for additional analysis in attempt to cover all possible variations of clinical samples sent to the laboratory. ESR values analyzed by both methods were compared and analyzed for its correlation, proportional difference, and bias.

**Statistical analysis**

For precision study, mean, SD and CV were calculated. A paired sample t-test and Wilcoxon signed-rank test were used for comparison of groups and p<0.05 was considered statistically significant. Linear regression was calculated using Passing–Bablok regression analysis. The nonparametric test of Spearman’s rank correlation was used to evaluate correlation (r, correlation coefficient). Bias and 95 % limits of agreement assessments were performed using Bland–Altman analysis. The calculations were performed by using MedCalc statistical software Version 20.2.18 (Ostend, Belgium) and GraphPad Software Version 9.5.1 (La Jolla, CA, USA).

**Results**

**Precision**

The results of intra-run and inter-run studies are presented in Table 1. The intra-run study using patient samples was measured in 10 replicates and yielded CVs ranging from 3.07 to 17.02 %. Lower ESR values tend to show a higher CV than higher ESR values. In the inter-run study, measured values of 3 levels of QC materials were within the target values with low CV (ranging from 0.68 to 1.65 %). All precision data were within the manufacture’s claimed values [14].

**Effect of potential interferences**

Both hemolysis and lipemic interferences significantly increased ESR levels (Table 2). Mean bias were 14.46 mm (95 % CI: 7.71 to 21.21) and 6.98 mm (95 % CI: 1.96 to 11.99) for hemolysis and lipemic interferences, respectively. By using healthy volunteer samples, increasing fibrinogen concentration led to an increase in ESR values. The Westergren method tended to show higher fibrinogen sensitivity than BC-780 in the sample with high ESR baseline value (Figure 1).

**Carryover**

The carryover was analyzed from three consecutive runs of HTV follow by three consecutive runs of LTV. The average...
Table 1: Intra-run and inter-run precision studies of BC-780 automated analyzer using patient samples and QC materials.

<table>
<thead>
<tr>
<th>ESR range, mm</th>
<th>Intra-run study using patient samples</th>
<th>Inter-run study using QC materials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (0–40)</td>
<td>Medium (40–80)</td>
</tr>
<tr>
<td>Sample no.</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ESR value</td>
<td>4.85 ± 0.83</td>
<td>18.41 ± 1.71</td>
</tr>
<tr>
<td>mean ± SD, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV, %</td>
<td>17.02</td>
<td>9.27</td>
</tr>
</tbody>
</table>

ESR, erythrocyte sedimentation rate; SD, standard deviation; CV, coefficient of variation. Manufacturer’s claimed values: ESR 0–20 mm ≤1.8 (SD), ESR>20 mm ≤9 % (CV).

Table 2: Evaluation of hemolysis and lipid interferences.

<table>
<thead>
<tr>
<th>Hemolysis (n=14)</th>
<th>Lipemic (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-hemolysed</td>
</tr>
<tr>
<td></td>
<td>Non-lipemic</td>
</tr>
<tr>
<td>Median, mm (min-max)</td>
<td>31.91 (3.07–113.58)</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean bias, %</td>
<td>57.87</td>
</tr>
<tr>
<td>Mean bias, mm (95 % CI)</td>
<td>14.46 (7.71–21.21)</td>
</tr>
</tbody>
</table>

CI, confidence interval, <sup>a</sup>p<0.05 was considered statistically significant.

carryover from 3-set of HTV and LTV was 0.49 % (95 % CI: −3.05 to 4.04) which was within the manufacturer’s claim of <1 %.

**Sample stability**

Sample stability at RT (1st experiment) revealed that ESR values did not change significantly (p=0.436) after being stored at RT for 8 h. However, when the samples were kept at RT for a longer period, up to 24 h from venipuncture, the ESR values decreased significantly (p<0.001) when compared to storage at RT for both <4 and 8 h periods. Refrigerated samples at 2–8 °C until 24 h from venipuncture (2nd experiment), showed unchanged ESR values when compared with fresh specimens (p=0.898). The 3rd experiment was set to simulate the real routine practice in which samples would be kept in both RT and refrigerated conditions. ESR values were consistently unchanged (p=0.096) after being stored at RT for 8 h. Samples were then refrigerated up to 24 h from

Figure 1: Sensitivity to fibrinogen: ESR levels were measured after addition of various concentration of fibrinogen. Four healthy volunteer samples were analyzed by (A) BC-780 and (B) Westergren method. Fibrinogen concentration in working solution is indicated on y-axis.
venipuncture. Reanalysis of ESR values demonstrated that the later refrigeration of samples (at 8 h from venipuncture) led to a significant reduction in ESR values when compared to fresh specimens (p=0.001) and specimens stored for 8 h at RT (p=0.017) (Table 3).

### Method comparison

The median ESR value of 198 samples (with normal CBC results) measured by BC-780 was 26 mm (Interquartile range (IQR): 10.00 to 45.02) which was significantly higher (p<0.001) than the value of 21 mm (IQR: 9.00 to 45.50) measured by Westergren reference method. Subgroup analysis according to ESR ranges demonstrated that BC-780 yielded significantly higher ESR values (p<0.001) than Westergren reference method only in the low ESR range group (ESR<40 mm/h), n=144. Comparable ESR values between two methods were observed in medium and high ESR range groups with p=0.262 and p=0.256, respectively (Table 4). Passing–Bablok linear regression yielded proportional difference with a regression equation: 3.08+0.98x. The obtained Spearmen’s rank correlation coefficient (r) was 0.901 (95% CI: 0.871 to 0.924) (Figure 2A). Bland–Altman analysis showed a positive bias of BC-780 of 3.12 mm (95% CI: 1.28 to 4.96) (Figure 2B).

As shown in Table 5, additional analysis of ESR values was also performed in blood samples with varying levels of Hct and MCV. Most results showed that BC-870 yielded significantly higher (p<0.01) ESR values than the Westergren method in all Hct and MCV ranges. However, mean absolute biases were limited to 4.80–4.84 for both Hct<35 % and Hct≥35 % groups. Similarly, mean absolute bias of MCV<80 fl, 80–100 fl and >100 fl groups were 2.60, 4.70 and 7.99, respectively.

### Discussion

Automation of ESR measurement is currently an attractive proposition. However, validation and comparison against

---

**Table 3:** Sample stability study.

<table>
<thead>
<tr>
<th>Storage time</th>
<th>ESR (median, mm)</th>
<th>ESR (IQR)</th>
<th>p-Value</th>
<th>Mean bias, %</th>
<th>Mean bias, mm (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4 h (1)</td>
<td>24.11 (12.65–37.22)</td>
<td>&lt;0.001* (1/2)</td>
<td>-0.73 (1/2)</td>
<td>0.65 (-0.92 to 2.22) (1/2)</td>
<td></td>
</tr>
<tr>
<td>8 h (2)</td>
<td>24.54 (12.36–41.88)</td>
<td>&lt;0.001* (1/2)</td>
<td>0.87 (1/2)</td>
<td>6.21 (3.75–8.68) (1/2)</td>
<td></td>
</tr>
<tr>
<td>24 h (3)</td>
<td>19.71 (19.43–34.37)</td>
<td>&lt;0.001* (2/3)</td>
<td>2.75 (1/3)</td>
<td>5.56 (4.02–7.10) (2/3)</td>
<td></td>
</tr>
<tr>
<td>2–8 °C</td>
<td>-4 h (1)</td>
<td>25.80 (14.00–40.44)</td>
<td>0.898</td>
<td>-0.70 (-0.92 to 2.31) (2/3)</td>
<td>0.69 (-0.21 to 1.59) (4/5)</td>
</tr>
<tr>
<td>8 h (5)</td>
<td>21.98 (10.86–43.74)</td>
<td>0.001* (4/6)</td>
<td>0.69 (1/3)</td>
<td>3.22 (1.06–5.38) (4/6)</td>
<td></td>
</tr>
<tr>
<td>24 h (6)</td>
<td>19.16 (11.56–45.74)</td>
<td>0.017* (5/6)</td>
<td>3.22 (1/3)</td>
<td>7.18 (5/6)</td>
<td></td>
</tr>
</tbody>
</table>

IQR, inter quartile range; CI, confidence interval; *p<0.05 was considered statistically significant; (1) to (6) indicate the ESR values in different timepoints and storage conditions.

**Table 4:** Comparison of ESR values measured by Westergren and BC-780 analyzer in normal CBC samples.

<table>
<thead>
<tr>
<th>ESR range</th>
<th>ESR value, mm</th>
<th>p-Value</th>
<th>Mean absolute bias, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 (n=144)</td>
<td>13.00 (5–22)</td>
<td>&lt;0.001*</td>
<td>4.95 (3.58–6.33)</td>
</tr>
<tr>
<td>40–80 (n=32)</td>
<td>55.00 (50–68)</td>
<td>0.262</td>
<td>0.13 (-8.43–8.16)</td>
</tr>
<tr>
<td>&gt;80 (n=22)</td>
<td>96.50 (89–121)</td>
<td>0.256</td>
<td>4.82 (-2.58–12.21)</td>
</tr>
<tr>
<td>All (n=198)</td>
<td>21.00 (9–45.5)</td>
<td>&lt;0.001*</td>
<td>3.12 (1.28–4.96)</td>
</tr>
</tbody>
</table>

*ESR ranges were set according to the values measured by the Westergren method. *ESR (inter quartile range). *Wilcoxon signed-rank test was used to compare the values obtained from Westergren and BC-780. *Mean absolute biases were calculated according to the formula: bias=BC-780 – Westergren. Absolute bias is presented as mean and 95 % CI. The allowable bias is set to 5 mm. *p<0.05 was considered statistically significant.
MCV levels \( \geq \) signed-rank test were used to compare the values obtained from Westergren and BC-

Hct levels \( \leq \) significant.

\[ Y = 3.079 + 0.982X \]

Bablok scattergram with an equation \( y = 3.079 + 0.982x \) and slope 0.982 (95 % CI: 0.871 to 0.924).

(B) Bland–Altman plot with mean bias of 3.12 (95 % CI: 1.277 to 4.962) is shown with \( \pm 2.50 \).

Figure 2: Method comparison study of ESR values analyzed by BC-780 vs. the Westergren reference method using 198 normal CBC samples. (A) Passing–Bablok scattergram with an equation \( y = 3.079 + 0.982x \) and slope 0.982 (95 % CI: 0.871 to 0.924).

(B) Bland–Altman plot with mean bias of 3.12 (95 % CI: 1.277 to 4.962) is shown with \( \pm 2.50 \).

Table 5: Comparison of ESR values measured by Westergren method and BC-780 analyzer in different Hct and MCV levels.

<table>
<thead>
<tr>
<th>Hct and MCV levels</th>
<th>ESR value, mm(^b)</th>
<th>p-Value(^c)</th>
<th>Mean absolute bias(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 35 % ) (n=210)</td>
<td>35.50 (11.00–64.25)</td>
<td>41.07 (25.75–63.43)</td>
<td>(&lt;0.001^a) 4.84 (2.63–7.06)</td>
</tr>
<tr>
<td>( \geq 35 % ) (n=176)</td>
<td>12.00 (5.00–22.75)</td>
<td>18.96 (8.95–30.37)</td>
<td>(&lt;0.001^a) 4.80 (3.67–5.92)</td>
</tr>
<tr>
<td>MCV levels ( \leq )</td>
<td>( &lt;80 ) fl (n=146)</td>
<td>16.50 (8.75–28.25)</td>
<td>20.51 (12.13–33.36)</td>
</tr>
<tr>
<td>( &gt;80 ) fl (n=194)</td>
<td>26.50 (11.00–52.50)</td>
<td>36.21 (18.61–58.50)</td>
<td>(&lt;0.001^a) 4.70 (2.90–6.41)</td>
</tr>
<tr>
<td>( &gt;100 ) fl (n=51)</td>
<td>26.00 (13.00–42.00)</td>
<td>39.66 (25.08–53.77)</td>
<td>(&lt;0.001^a) 7.99 (4.57–11.42)</td>
</tr>
</tbody>
</table>

\( ^a \) Hct and MCV values were obtained from BC-780 automated hematology analyzer using CBC + DIFF + ESR mode. \( ^b \) Median (interquartile range). \( ^c \) Wilcoxon signed-rank test were used to compare the values obtained from Westergren and BC-780. \( ^d \) Mean absolute biases were calculated according to the formula: bias = BC-780 – Westergren. Absolute bias is presented as mean and 95 % CI. The allowable bias is set to 5 mm. \( ^a \) p<0.05 was considered statistically significant.

the Westergren reference method is required for both alternate and modified ESR measurement methods. Test results are varied as they employ different methods of detection, phases of detection, or anticoagulants used. For example, Čičak et al., reported differences in test performance and bias of two automated analyzers (Roller 20 PN and iSED) when compared to the Westergren method which cannot be used interchangeably [5]. Previously, we have also compared two modified Westergren analyzers (MIXRATE® X20 and VISION A) that uses different types of blood tubes and anticoagulants. Results showed that, although the correlation of both analyzers was very good at low and ESR levels, systemic biases were documented at high ESR levels [9].

Here, we described the performance of the BC-780 automated hematology analyzer that can simultaneously analyze ESR together with CBC. The precision study showed results consistent with previous reports. Samples with low ESR values tend to show higher CV when analyzed by either alternate (Test 1 and iSED) [17, 21] or modified Westergren analyzers (Ves-Matic Cube 200, MIXRATE® X20 and VISION A) [9, 17]. Despite these differences, all precision results analyzed by BC-780 passed the manufacturer’s claim [14].

No obvious carryover was detected from carryover studies. Mean carryover found in this study agreed with the previous study using similar model of analyzer, BC-720 which yielded carryover of less than 1 % and met the manufacturer’s claim [22]. The carryover obtained in this study...
was lower than previous studies which also used alternate ESR analysis methods including Test 1 and iSED. Those studies reported a potential carryover of around 2.9–4.2% [10, 17, 23]. However, for those analyzers, the detected carryover was still lower than the instrument’s CV and might not affect the interpretation of results.

As expected, increase in positively charged plasma proteins like fibrinogen resulted in higher ESR levels. This is consistent with most previous studies using conventional Westergren, modified Westergren, and other alternate methods, as positively charged proteins including fibrinogen, c-reactive protein, etc., is known to enhance rouleaux formation, thus enhancing the sedimentation rate [9, 16, 17, 24]. Interestingly, in this study, BC-780 seems to show less sensitivity to fibrinogen than the Westergren method as seen in sample#4 which had a higher ESR baseline than others. Similar to the previous study conducted using the Test 1 analyzer, it was also less affected by fibrinogen level than the Westergren method [17]. However, further investigation of fibrinogen sensitivity in clinical samples with various ESR baseline is crucial to confirm our findings.

To our knowledge, this was the first study of potential interferences in ESR measurement using the BC-700 series. We found that both hemolysis and lipemic samples significantly increased ESR values. This contrasted with most previous studies which suggested that hemolysis usually reduces the ESR results as it could interfere with rouleaux formation [9, 16, 17, 24]. Interestingly, this study, BC-780 seems to show less sensitivity to fibrinogen than the Westergren method as seen in sample#4 which had a higher ESR baseline than others. Similar to the previous study conducted using the Test 1 analyzer, it was also less affected by fibrinogen level than the Westergren method [17]. However, further investigation of fibrinogen sensitivity in clinical samples with various ESR baseline is crucial to confirm our findings.

High sample stability (up to 8 h at RT and up to 24 h at 2–8 °C) when analyzed by BC-780 could provide great benefit in routine laboratory testing in which delay of analysis can occur during the peak hours or when repeat/rerun is requested (by using stored sample). However, delayed sample refrigeration (>8 h from venipuncture) must be avoided as the ESR value will be decreased. This is similar to another finding that used EDTA-anticoagulated blood and was analyzed by iSED. It demonstrated that the measured values did not significantly change after being stored for 4 h, 8 h, 12 h and 24 h at both RT and 4–8 °C [23]. This might be due to EDTA’s excellent cell morphology preservation property during the storage period. Also, some prior studies reported that citrated anticoagulated blood altered ESR values if the measurement is delayed [2, 27]. However, this is in contrast to the findings from Brencic et al., which found that storage of EDTA samples for 3 h at room temperature yielded a significant increase in ESR values when measured by iSED [28]. Thus, to comply with ICSH guidelines and to ensure the reliability of results, ESR analysis should be performed as soon as possible.

The majority of samples (73% or 144 out of 198) used in the correlation study was categorized into the low ESR range. Significantly higher ESR values measured by BC-780 in low ESR range may result in a significantly higher ESR values compared to the Westergren reference method when all sample were analyzed. Moreover, it is worth exploring whether these different values would yield a difference in the interpretation of results. In our study, 0.7% (13/198) of the samples had a different values would yield a difference in the interpretation of results. In our study, 0.7% (13/198) of the samples had a different clinical interpretation, especially for the samples in low ranges measured by the Westergren method. Therefore, careful interpretation of results and establishment of a reference range specific to different alternate analytical method should be taken into consideration. In the previous report, Shu et al. demonstrated that the ESR reference interval of healthy Chinese individuals measured by BC-720 was <15 mm for males and <24 mm for females [22]. In the same study, they also showed that populations from different geographical regions had different normal ESR ranges. Hence, the establishment of a reference interval for a specific population before implementing an alternate ESR measurement method in a clinical laboratory is warranted. Similar to our study, previous studies using other alternate ESR measurement analyzers, including iSED and Test 1, reported a difference in the obtained values when compared with the Westergren method. The mean bias obtained from iSED and Test 1 when compared with the Westergren reference method was 8.1–13 mm [10, 25] and 1.1–7.3 mm [29], respectively. However, in our study, even after analyzing according to the ESR range or analyzing all 198 samples, the mean absolute bias obtained was still within the acceptable bias criteria of 5 mm as recommended by ICSH guidelines [13].

In addition to normal CBC samples, the method comparison study was extended to samples with different Hct and MCV values which has been shown to affect the value analyzed by the Westergren method [7, 19, 20]. When data were analyzed according to Hct levels, it was shown that BC-780 is less affected by Hct and MCV values. Only sample with MCV>100 FL showed the high mean absolute bias value
(>5 mm). However, only 51 samples with MCV>100 fL were enrolled in this study. A larger sample size might be required to confirm this finding in future.

In conclusion, BC-780 automated hematology analyzer showed high correlation with the reference Westergren method with an acceptable positive bias and was less effect by varying Hct and MCV levels. EDTA blood samples were stable up to 8 h at RT and up to 24 h at 2–8 °C. Delay of sample refrigeration (>8 h) should be avoid. Precision, and carry-over studies agreed with the manufacturer’s claims. However, further intensive validation using real clinical sample against the level hemolysis/lipemic that could interfere the test results is warranted.

Acknowledgments: We would like to thank Ms. Sunudda Nowaratsopon, Mr. Suppakorn Wongkamchai, Ms. Waroonkarn Laiklang, Ms. Pitchayaporn Yiyanggoon, and Ms. Piyada Kaewkanya for blood samples collection. We are also grateful to Mr. Kosai Yuyim for hemoglobin and triglyceride concentration analysis. We thank Firmer Co Ltd for supporting BC-780 instrument and reagents.

Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Committee of Faculty of Medicine, Chulalongkorn University (approval number 1689/2022).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

Research funding: None declared.

Data availability: The raw data can be obtained on request from the corresponding author.

References


