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Performance evaluation of a novel platelet count parameter, hybrid platelet count, on the BC-780 automated hematology analyzer

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Abstract

Objectives: Automated hematology analysis is expected to improve the performance of platelet counting. We evaluated the performance of a new platelet counting, hybrid (PLT-H) and also impedance (PLT-I) and optical (PLT-O) on the BC-780 automated hematology analyzer compared to the international reference method (IRM) in blood samples with thrombocytopenic and platelet interference.

Methods: The basic platelet count performance of the BC-780 automated hematology analyzer was evaluated according to the requirements of the Clinical Laboratory and Standards Institute (CLSI) Document H26-A2. Additionally, the thrombocytopenic (low PLT count) blood samples and the platelet interference blood samples including fragmented red blood cells (RBCs), microcytes or small RBCs, and giant platelets were determined with the BC-780 hematology analyzer compared to the IRM.

Results: Blank counting and the carry-over contamination rate of platelet count using the BC-780 both met the manufacturers' claim. For both 123 thrombocytopenic and 232 platelet interference blood samples (72 fragmented RBCs, 91 microcytes and 51 giant platelets), all three platelet counting methods exhibited high comparability with the IRM (the lowest correlation (r)=0.916). Interestingly, the comparability of PLT-H (r =0.928–0.986) with the IRM was better than that of PLT-I (r =0.916–0.979).

Conclusions: The performance of PLT-H in the BC-780 met the manufacturer's specifications. PLT-H exhibits better

reproducibility than did PLT-I, correlates well with the PLT-O for thrombocytopenic samples and demonstrates good anti-interference ability. PLT-H counting is therefore recommended as a zero-cost alternative platelet counting method for platelet interference samples in clinical settings.

Keywords: hybrid platelet count (PLT-H); international reference method; thrombocytopenia; platelet interferences; evaluation

Introduction

Accurate platelet count plays a vital role in the diagnosis and management of bleeding, particularly when considering platelet transfusion. It is essential to obtain accurate platelet counts to ensure appropriate treatment and intervention for patients experiencing these conditions. There are several methods for determining platelet count, including microscopic examination, automated hematology analyzers, and the international reference method (IRM) based on flow cytometry, which is recommended by the International Council for Standardization in Hematology (ICSH) [1] and the International Society of Laboratory Hematology (ISLH) [2]. The microscopic examination involves a manual platelet count on a peripheral blood smear under a microscope. It is routinely used to review platelet counts, especially when thrombocytopenia or other platelet-interfering factors such as fragmented red blood cells (RBCs) or small RBCs and giant platelets are present. While manual microscopy provides a visual and specific approach, this time-consuming technique is prone to imprecision and poor reliability [3]. Immunofluorescence detection by flow cytometry is the international reference method; however, its application in clinical laboratories is limited due to various factors, including the high cost associated with reagents and instruments, challenges in standardization, labor-intensive requirements, and the need for highly skilled and competent personnel, which makes it less feasible when compared to modern automated hematology analyzers.

The automated hematology analyzers use various platelet count methods, each with its distinct advantages and limitations [4]. The impedance method (PLT-I) is most widely

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used as the primary method for platelet counting; however, it has specific limitations [5]. Due to the use of high or low pulses to identify large or small particles, PLT-I cannot distinguish particles that are similar in size to platelets [6]. In clinical practice, various interfering factors can lead to inaccuracies in PLT-I count; for example, small RBCs, fragmented RBCs, and fragmented WBCs can cause falsely increased platelet counts; on the other hand, platelet aggregation and giant platelets can cause falsely decreased platelet counts [7]. To overcome the limitations of PLT-I, several manufacturers have developed additional methods to provide accurate verification of platelet count. The Abbott CELL-DYN series, Siemens ADVIA series, and other systems have implemented the optical method (PLT-O) for platelet counting. Although this method improves the detection of larger platelets, it still has a weakness in effectively addressing interference caused by small RBCs or cellular debris [7]. The BC-700, BC-6000 and BC-6800 series (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) and the XE and XN series (Sysmex, Kobe, Japan) have been developed on the basis of the fluorescent optical method. The incorporation of nucleic acid fluorescent dyes provides a better platelet identification which helps minimize interference from small RBC and cell debris [8]. This capability is particularly advantageous when dealing with low platelet counts [9]. Currently, this fluorescent optical method is being applied in clinical practice when platelet interference is suspected by the PLT-I method. However, this method requires new independent physical channels (RET-channel) and the use of additional reagents. It is crucial to consider the additional expense associated with this implementation.

A new zero-cost platelet count parameter, hybrid (PLT-H), is developed in the BC-700 series (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). The PLT-H method is a combination platelet count algorithm between the small platelet count derived from the impedance channel and the large platelet count derived from the DIFF channel, which helps to reduce platelet interference.

In this study, we evaluated the performance of different platelet counting methods: PLT-I, PLT-O, and a new PLT-H on BC-780 automated hematology analyzers by comparing with the IRM in blood samples with thrombocytopenia and platelet interference.

Materials and methods

Study blood samples

The leftover whole blood samples were obtained from the inpatient and outpatient departments, at King Chulalongkorn Memorial Hospital

using inclusion and exclusion criteria from March to July 2022. All samples were collected in dipotassium ethylenediaminetetraacetic acid (K₂-EDTA) (Becton-Dickinson, NJ, USA) and analyzed using different platelet counting methods within 4 h after blood collection. Samples with flags suggesting platelet clumps were excluded. This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (approval number 756/63). As this study was conducted using leftover blood samples from services, the requirement for obtaining written informed consent was waived.

For thrombocytopenic blood samples, samples without platelet interfering factors were divided into two groups according to different levels of clinical-related bleeding risks: $\leq 50 \times 10^9/L$ ($n=77$), and $\leq 100 \times 10^9/L$ ($n=123$). For further investigation of the impact of inferences on impedance platelet counting, three groups of samples were used: (i) fragmented RBCs: samples with the percentage of fragmented RBCs (FRC%) $>2\%$ ($n=72$), (ii) microcytes: samples with mean corpuscular volume (MCV) less than 65 fL ($n=92$) and (iii) giant platelets: samples with platelet diameter $>7 \mu m$ and microscopic platelet count $>5/200$ WBCs (result obtained from the MC-80 automated digital cell morphology (Mindray, Shenzhen, China)) ($n=68$). All interference samples were included based on the criteria that required samples to be flagged for each specific interference by an automated hematology analyzer and subsequently verify through examination of a blood smear.

Performance evaluation of different platelet count methods

The basic performance of different platelet count methods on the BC-780 automated hematology analyzer was evaluated according to the Clinical Laboratory and Standards Institute (CLSI) Document H26-A2 [10].

- **Background counting:** The diluent was run on the analyzer three times, and the maximum of the three results was used as the background count.
- **Carryover:** A high concentration with platelet $>900 \times 10^9/L$ sample was run three times and recorded as H1, H2, and H3, respectively. Then, a low concentration with platelet $<30 \times 10^9/L$ sample was run three times and recorded as L1, L2, and L3, respectively. The carryover was calculated according to the formula: $\text{carryover (\%)} = [(L1 - L3)/(H3 - L3)] \times 100\%$.
- **Reproducibility for low platelet count:** The reproducibility of different platelet count methods on BC-780 was evaluated in low platelet count samples. Eight and Seven samples from each range, including <50 and $51-100 \times 10^9/L$ were selected and repeatedly run 10 times. The mean, standard deviation (SD), and coefficient of variation (%CV) were calculated.

Comparison of different platelet count methods with the international reference method

According to the manufacturer's instructions, a total of 123 thrombocytopenic samples (77 had PLT counts less than $50 \times 10^9/L$ and 46 had PLT counts $51-100 \times 10^9/L$) and three types of platelet interfering samples including 72 fragmented RBCs, 92 microcytes, and 68 giant platelet samples were analyzed using the Mindray BC-780 hematology analyzer in CBC+DIFF+RET (CDR) mode. BC-780 provided three platelet counts: impedance (BC780 PLT-I), fluorescent optical (BC780 PLT-O) and hybrid (BC780 PLT-H).

In the BC-700 series, the new PLT-H parameter is reported in every CBC+DIFF (CD) mode without requiring additional reagents. The PLT-H method is a mathematical data manipulation, combining the small platelets measured in the conventional impedance method with larger platelets that are detected in the WBC differential channel. In contrast, PLT-I relies solely on impedance measurement using hydrodynamic focusing. Regarding PLT-O in CDR mode, the BC-700 series utilizes dedicated nucleic acid fluorescence dyes for platelet staining within the reticulocyte (RET) channel, ensuring accurate optical platelet count results with similar technology that employed in the BC-6000 and 6800 series. In case of low PLT counts, the instrument software will automatically extend the counting time of the PLT-O method 5-fold, which leads to enhanced precision.

The IRM or the immunoplatelet method was performed with CD41-FITC and CD61-APC antibodies using the BD FACSCanto-II™ flow cytometer (Becton Dickinson, BD Biosciences, CA, USA), according to the ICSH and ISLH guidelines [2]. Briefly, 5 μ L of the blood sample was mixed with 100 μ L of phosphate buffer saline with bovine serum albumin (PBS-BSA), 5 μ L of anti-CD41 antibody and 5 μ L of anti-CD61 antibody, then incubated at room temperature for 15 min in the dark. Finally, the samples were diluted to 1:1,000 by adding 4.85 mL of PBS-BSA. For the flow cytometry analysis, at least 50,000 RBC events or 1,000 platelet events were counted. The platelet count ($\times 10^9$ /L) or FACS PLT was finally calculated using the formula below.

$$\text{Platelet count } (\times 10^9/\text{L}) = \text{RBC count } (\times 10^{12}/\text{L}) \times \frac{\text{platelet gated events}}{\text{RBC gated events}}$$

The RBC count used in this formula was measured with BC-780 automated analyzers (impedance).

Statistical analysis

Statistical analysis was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guideline for Measurement Procedure Comparison and Bias Estimation Using Patient Sample (EP09-A3) [11]. The Pearson correlation coefficient was performed to assess the correlation between three different platelet counting methods and IRM (the reference method). The consistency between the three platelet counting methods and IRM was individually assessed by Passing-Bablok regression and Bland-Altman analysis. Along with the Bland-Altman plot, the outliers between the methods can be identified. The CLSI guideline (EP09-A3) recommends employing the median, rather than the mean, as the preferred estimate of bias for both comparisons because of the skewness in the distribution of differences [11].

Data analysis was performed using Excel (Microsoft, Redmond, WA) and MedCalc® Statistical Software version 22.007 (MedCalc Software Ltd., Ostend, Belgium; <http://www.medcalc.org>; 2023).

Results

Background counting

The background of three different automated platelet count results (PLT-I, PLT-O, and PLT-H) in the BC-780 was 0.00 in all samples, which met the manufacture's claimed values [12].

Carryover

The carryover rates for PLT-I, PLT-O, and PLT-H in the BC-780 were all lower than 0.5 %, which was within the manufacturer's claim of <1 % (Table 1).

Reproducibility for low platelet counting

As shown in Table 2, the reproducibility of low platelet counting was verified based on different levels of clinical-related bleeding risks. The CV (%) increases as platelet count decreases and experiences a significant decline when platelet count falls below 20×10^9 /L (case 1–5 in Table 2). In addition, among the different platelet count methods, the PLT-O demonstrates exceptional reproducibility in detecting low platelet samples, with a significantly reduced robust CV (%) compared to both PLT-I and PLT-H.

Comparability analysis between different platelet counting methods and the IRM in the determination of thrombocytopenic samples

Two groups of thrombocytopenic samples (77 samples had PLT counts less than 50×10^9 /L and 46 samples had PLT counts ranging from 51 to 100×10^9 /L) were separately analyzed in the BC-780 for the PLT-I, PLT-O, and PLT-H and in the flow cytometry for IRM. The Pearson correlation for the thrombocytopenic samples in both PLT ranges (<50 or $51\text{--}100 \times 10^9$ /L) showed that PLT-H is comparable to PLT-O ($r=0.956$ and 0.928 , respectively) and higher than PLT-I ($r=0.927$ and 0.916 , respectively) (Table 3). Passing-Bablok regression analysis showed that compared to IRM, the intercepts and the slopes for the three platelet count methods in the group with PLT count $<50 \times 10^9$ /L were all close to 0 and 1, respectively. Within the group with PLT count ranging from 51 to 100×10^9 /L, all three methods displayed slopes that were approximately equal to 1. However, the intercepts for the PLT-O and PLT-H exhibited a slight increase (1.458 and 1.366, respectively), while for the PLT-I, there was a

Table 1: Carryover contamination results in the BC-780.

	High value			Low value			Carryover
	H1	H2	H3	L1	L2	L3	
PLT-I	844	829	848	13	14	11	0.24 %
PLT-O	930	890	905	26	25	26	0.00 %
PLT-H	853	837	857	18	22	19	0.12 %

Table 2: Reproducibility results of low platelet counting by three different platelet count methods in BC-780.

PLT range, $\times 10^9/L$	Case	PLT-I			PLT-H			PLT-O		
		Mean, $10^9/L$	SD	%CV	Mean, $10^9/L$	SD	%CV	Mean, $10^9/L$	SD	%CV
<50	1	19.78	3.77	19.05	15.67	1.32	8.44	17.22	0.67	3.87
	2	19.67	3.28	16.67	17.67	1.80	10.20	20.56	0.53	2.56
	3	9.89	1.27	12.84	10.11	1.36	13.49	11.67	0.50	4.29
	4	9.11	1.90	20.86	11.78	1.40	11.84	11.56	0.53	4.56
	5	8.33	1.50	18.00	8.89	1.36	15.35	5.33	0.50	9.38
	6	14.00	1.50	10.71	23.78	1.40	5.86	27.33	0.71	2.59
	7	38.67	2.24	5.78	39.78	1.99	4.99	38.33	1.00	2.61
	8	43.44	3.64	8.39	43.89	3.65	8.33	44.11	0.93	2.10
51–100	9	53.56	2.55	4.77	53.56	2.19	4.08	50.22	0.67	1.33
	10	60.11	4.65	7.73	61.44	3.75	6.10	59.78	0.83	1.39
	11	65.33	2.50	3.83	57.89	2.31	4.00	62.44	2.88	4.61
	12	62.22	4.41	7.09	62.67	3.39	5.41	65.33	1.73	2.65
	13	89.78	2.59	2.88	90.78	2.59	2.85	90.00	1.94	2.15
	14	88.00	3.35	3.81	89.11	3.37	3.78	85.56	2.00	2.35
	15	77.00	3.87	5.03	77.56	3.58	4.61	80.11	1.54	1.92

Gray background box table indicates less than the desirable specification of precision (7.6 %CV) according to the EFLM Biological Variation Database [13]. SD, standard deviation; CV, coefficient of variation.

Table 3: Comparability analysis between the BC780 and the IRM in assessing thrombocytopenic samples.

Platelet range, $\times 10^9/L$	Parameter	Correlation coefficient, r	Passing-Bablok regression analysis		Bland-Altman analysis
			Intercept (95 % CI)	Slope (95 % CI)	Median (95 % CI)
<50 (n=77)	PLT-I	0.927	−0.393 (−2.867 to 1.353)	1.108 (1.032–1.205)	1.560 (0.820–3.140)
	PLT-O	0.978	0.551 (−0.358 to 1.399)	1.037 (0.995–1.088)	1.250 (0.970–1.730)
	PLT-H	0.956	−0.345 (−1.729 to 0.928)	1.029 (0.973–1.095)	0.450 (−0.960 to 1.310)
51–100 (n=46)	PLT-I	0.916	−2.239 (−14.618 to 8.070)	1.087 (0.931–1.242)	2.975 (0.400–5.500)
	PLT-O	0.941	1.458 (−2.868 to 7.879)	1.014 (0.853–1.089)	2.340 (0.100–4.090)
	PLT-H	0.928	1.366 (−8.475 to 7.840)	0.976 (0.885–1.095)	−0.390 (−3.400 to 0.400)

CI, confidential interval; IRM, international reference method.

decrease (−2.239) (Figures 1, 2, and 3). The Bland-Altman bias analysis showed that PLT-H has a minimal median difference with the IRM and has the best 95 % CI of the bias (Figure 3).

Comparability analysis between different platelet counting methods and the IRM in the determination of platelet interference samples

Based on different types of platelet interfering factors, the samples were divided into three groups: 72 fragmented RBCs, 92 microcytes, and 68 giant platelet samples. All samples were analyzed in the BC-780 for PLT-I, PLT-O, and PLT-H and in the flow cytometry for IRM. The results of Pearson's correlation, Passing-Bablok regression analysis, and Bland-Altman bias analysis (Table 4 and

Figures 1, 2, and 3) showed that the three different platelet counting methods had satisfactory comparability with IRM. Furthermore, in the presence of fragmented RBCs and microcytes, the PLT-O showed the best correlation coefficient followed by the PLT-H, which is slightly different from the PLT-I. However, there was no difference in the correlation coefficient between PLT-H and PLT-I in the presence of giant platelets. Overall, the performance of PLT-H in the determination of platelet interference samples was better than that of PLT-I and is almost comparable to that of PLT-O.

Discussion

Accurate platelet counting is crucial in the diagnosis, treatment, and prognosis of hematologic diseases, sepsis or multiorgan disorder [14–16]. Decreased platelet counts can

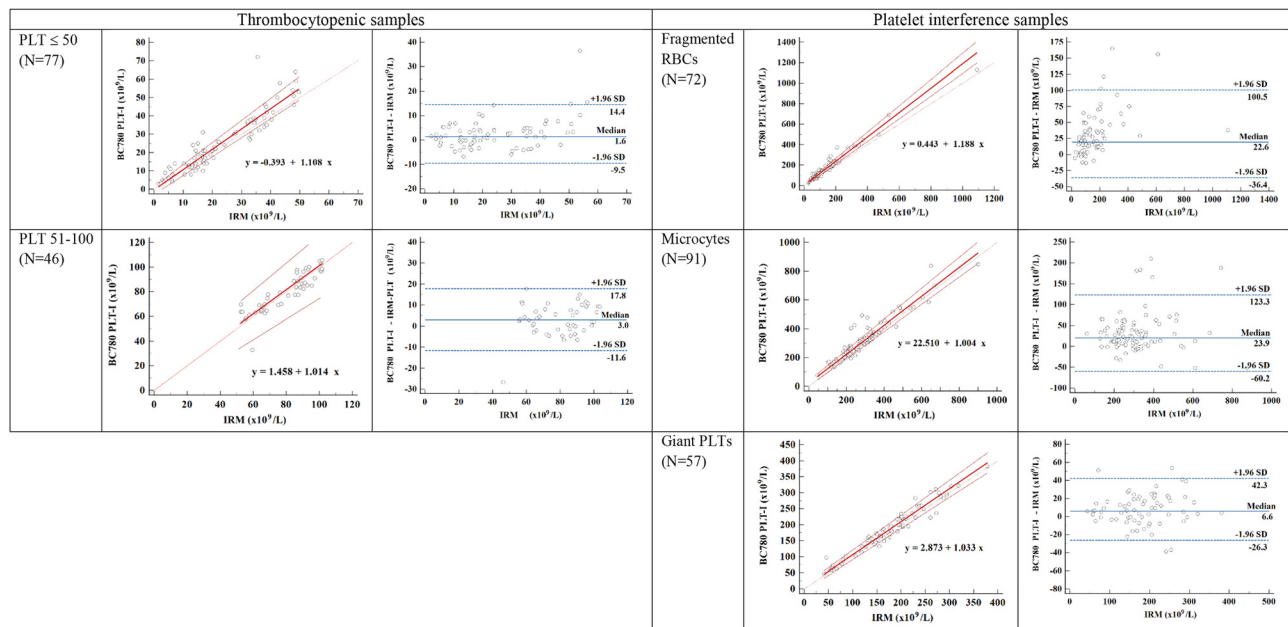


Figure 1: Passing-Bablok regression analysis and Bland-Altman analysis of PLT-I from BC-780 and the IRM. IRM, international reference method.

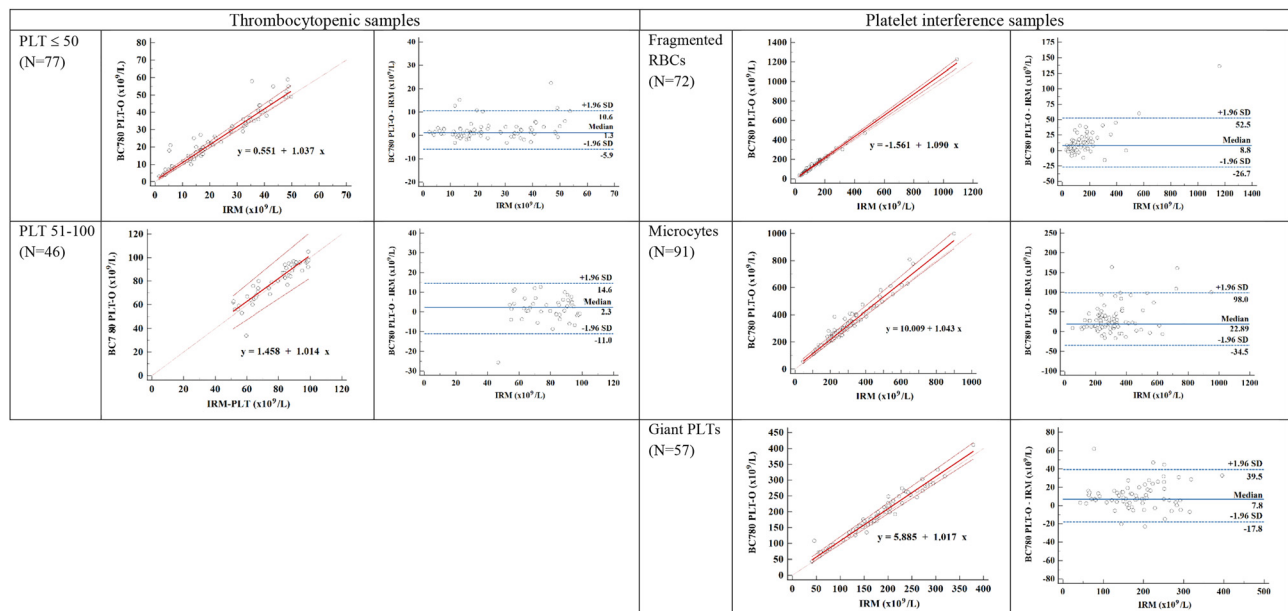


Figure 2: Passing-Bablok regression analysis and Bland-Altman analysis of PLT-O from BC-780 and the IRM. IRM, international reference method.

impact the speed of vascular repair and lead to bleeding [17], whereas elevated platelet counts can potentially promote the formation of blood clots [18]. As blood cell analysis technology progresses, there is an increasing clinical demand for platelet count methods that are faster, more accurate, and exceptionally precise. This is the first study that evaluated the performance of different platelet counting methods (impedance, fluorescent optical and hybrid) on

BC-780 hematology analyzers compared to the IRM in blood samples with thrombocytopenia and platelet interference. Furthermore, we explored the reproducibility of platelet counting at the low platelet counts.

The performance of the BC-780 hematology analyzer in background counting and carryover rate met the technical requirements. The reproducibility of low platelet count samples with three ranges, including <20 , $21-60$, and

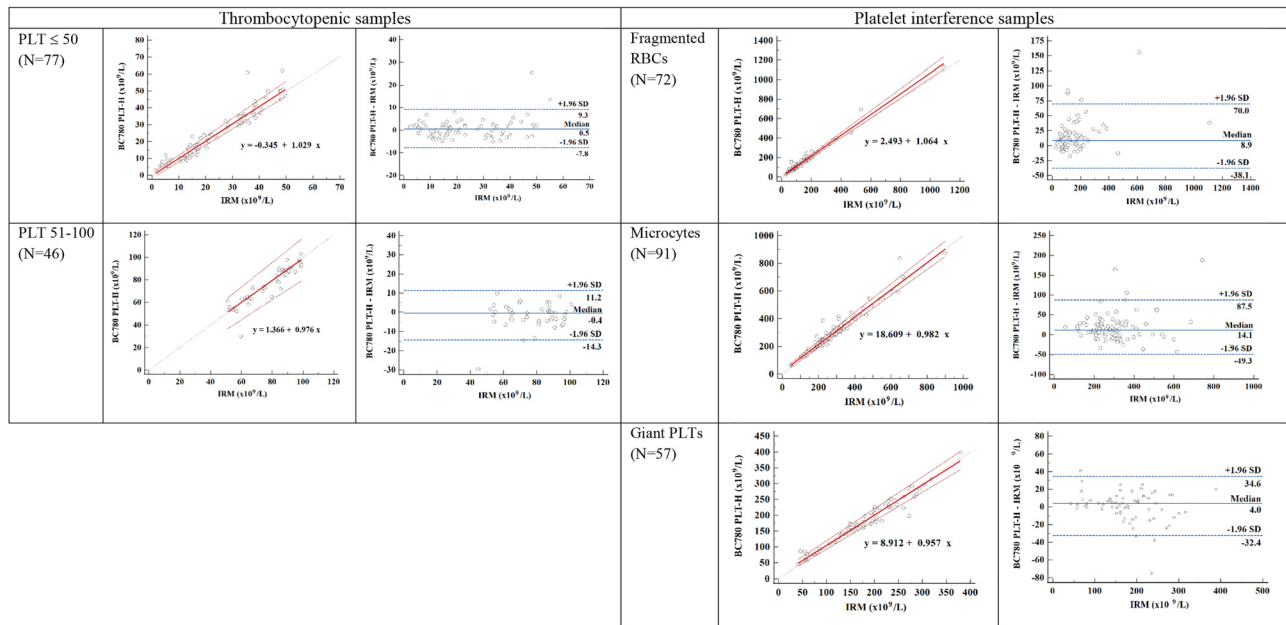


Figure 3: Passing-Bablok regression analysis and Bland-Altman analysis of PLT-H from BC-780 and the IRM. IRM, international reference method.

Table 4: Comparability analysis between the BC780 and the IRM in assessing platelet interference samples.

Interference samples	Parameter	Correlation coefficient, r	Passing-Bablok regression analysis		Bland-Altman bias
			Intercept (95 % CI)	Slope (95 % CI)	Median (95 % CI)
Fragmented RBCs (FRC>2 %) (n=72)	PLT-I	0.979	0.443 (−11.855 to 10.666)	1.188 (1.106–1.280)	22.585 (15.480–30.740)
	PLT-O	0.997	−1.561 (−5.200 to 3.972)	1.090 (1.048–1.124)	8.765 (4.200–12.840)
	PLT-H	0.986	2.493 (−7.833 to 6.899)	1.064 (1.022–1.124)	8.860 (2.990–12.950)
Microcytes (MCV<65 fL) (n=92)	PLT-I	0.944	22.510 (0.803–37.079)	1.005 (0.948–1.074)	23.930 (12.610–31.140)
	PLT-O	0.977	10.009 (−1.611 to 26.549)	1.043 (0.989–1.099)	22.890 (17.800–29.230)
	PLT-H	0.969	18.609 (5.169–30.299)	0.982 (0.938–1.034)	14.105 (10.020–19.690)
Giant platelets (n=68)	PLT-I	0.972	2.873 (−6.901 to 11.197)	1.033 (0.975–1.093)	6.640 (3.090–13.170)
	PLT-O	0.981	5.885 (−0.613 to 13.582)	1.017 (0.970–1.069)	7.800 (6.010–12.880)
	PLT-H	0.973	8.912 (2.629–18.536)	0.957 (0.901–1.010)	3.975 (−0.580 to 5.920)

CI, confidential interval; IRM, international reference method.

$61\text{--}100 \times 10^9/\text{L}$ found that PLT-O showed the best reproducibility in all three ranges, with a significantly lower robust CV (%) than PLT-H and PLT-I (Table 2). However, there is no difference among the three platelet counting methods when the platelet count is more than $60 \times 10^9/\text{L}$. Furthermore, the PLT-H results are comparable to PLT-O when the platelet count is $>20 \times 10^9/\text{L}$. This finding aligns with a previous study by Gioia M et al., where they observed that as platelet counts decreased, CV (%) increased in all nine different hematology analyzer [19]. To further verify the correlation between three platelet counting methods on BC-780 hematology analyzers and IRM, thrombocytopenic samples with two different ranges, $<50 \times 10^9/\text{L}$ and $51\text{--}100 \times 10^9/\text{L}$ were analyzed (Table 3). The correlation between PLT-H and the

IRM was found to be stronger than that of PLT-I and to be more comparable to that of PLT-O. Interestingly, the median bias and 95 % CI of PLT-H was less than PLT-O when compared with IRM for both two platelet count ranges. Our findings align with those of Sun et al. that the fluorescence optical method is more suitable for platelet count in thrombocytopenic patients compared to impedance methods [8]. Additionally, our results are consistent with the findings of Kim et al. that the enhanced PLT-O (PLT-O 8x) on the BC6000Plus (BC6000P) series is better than PLT-I in cases of thrombocytopenia [9]. Of note, the difference between the enhanced PLT-O mode in the BC-780 and BC6800P lie in the automatic extension of the counting time for samples with low platelet counts. Specifically, the BC-780 offers a 5-fold

increase, while the BC6800P provides an 8-fold increase. Furthermore, as the BC-780 is equipped with PLT-H, it has the capability to complement PLT-I results in case of thrombocytopenia.

In the presence of all three platelet interference factors, the PLT-O exhibited the highest accuracy for platelet counting compared to the IRM (Table 4). For interference from fragmented RBCs and microcytes, the PLT-H demonstrated a stronger correlation with the IRM than the PLT-I with a correlation coefficient greater than 0.969 and the bias of the PLT-H smaller than that of the PLT-I. However, there was no difference in the correlation between PLT-H and PLT-I with IRM when giant platelets were present. This could be due to the samples not being abnormal enough according to our current selection criteria, which focused on platelet diameter. In contrast, other study selected giant platelet samples based on the mean platelet volume [20].

This study has some limitations. First, our precision evaluation was limited in scope. We specifically focused on assessing the precision of low platelet levels on automated hematology analyzers, while neglecting to evaluate the precision of the IRM reference method. Second, we investigated the precision of platelet counts in thrombocytopenic samples. However, it is important to note that these results may not cover the entire range of platelet counts across various clinical conditions. Lastly, as mentioned previously, there is a possibility that the criteria used to select the giant platelet samples may not have been adequate.

This is the first study to evaluate the basic performance of a new PLT count technology, the PLT-H, on the BC-780 (Mindray, Shenzhen, China), a five-part hematology analyzer, and to evaluate its comparability with IRM in blood samples with thrombocytopenia and platelet interference. The results showed that the PLT-H on the BC-780 has (I) good essential functions in terms of background count and carryover; (II) high correlation with IRM in thrombocytopenic samples, (III) good anti-interference ability for small RBCs, fragmented RBCs and giant PLTs in comparison with IRM. Furthermore, PLT-H exhibits better reproducibility than PLT-I and correlates well with PLT-O. Therefore, PLT-H counting would be a promising alternative option with zero cost in blood samples with thrombocytopenic and platelet interference in clinical hematology laboratories, especially in low-to middle-income countries.

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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 756/63).

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

Competing interests: Authors state no conflict of interest.

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

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