Platelet Counting with the BD Accuri™ C6 Flow Cytometer

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White Paper

Contents

Abstract

The development of quick and precise methods to determine the absolute number or concentration of platelets in whole blood or in purified preparations has been a constraint in the field of hematology. Historically, laboratories performed such counts using a microscope and hemocytometer, but this method is slow and prone to error, especially in samples with few platelets. Flow cytometry can rapidly identify platelets and discriminate them from debris and red blood cells (RBCs), but counting the cells commonly requires adding counting beads to the sample, or separately determining the RBC count using a hematology analyzer, and then calculating the RBC/platelet ratio.

The BD Accuri™ C6 flow cytometer is a compact, digital, low-cost instrument. Its unique fluidics system, driven by peristaltic pumps, can compute sample volume and cell concentration directly, bringing the advantages of single-platform flow cytometric cell counts into the hands of small labs and individual researchers.

This white paper reports the validation of the BD Accuri C6 flow cytometer for counting platelets in whole human peripheral blood specimens using the RBC/platelet ratio International Reference Method (IRM) and by direct volume computation. The direct volume method requires no additional measurement of RBC or reference bead counts, and offers the added benefits of increased speed and reduced cost and complexity.

The results showed that, as expected, platelet counts obtained using the IRM on a BD Accuri C6 are functionally interchangeable with IRM counts on a BD FACSCalibur™ flow cytometer. Similarly, for platelet counts spanning the normal range, direct volume computation on the BD Accuri C6 is functionally interchangeable with the IRM on either instrument.

When analyzing samples with low platelet counts (<50 x 10^9/L), the direct volume method on the BD Accuri C6 showed a modest tendency to report higher counts than the IRM. For many studies, these counting differences will not affect results. In cases when it is important to accurately determine low platelet concentrations, it might be wise to validate results using the IRM as well.

Introduction

Platelets are small, discoid-shaped, anucleated cells derived in the bone marrow from precursor megakaryocytes during blood cell formation.[1] A typical healthy adult produces approximately 10^{11} platelets each day, which typically circulate for 10 days, resulting in a physiological range of 150–400 x 10^9/L. Of these, 30% are sequestered in the spleen and the remaining 70% circulate as quiescent cells surveying the integrity of the vasculature.

Upon detecting vessel-wall damage, platelets undergo explosive activation, when they release adhesive proteins, coagulation factors, and growth factors that facilitate communication with leucocytes and endothelial cells. They ultimately aggregate to form a "platelet plug" that occludes the site of damage, prevents blood loss, and preserves vascular integrity.[2] However, formation of the platelet plug in response to vascular damage can sometimes lead to vessel blockage (thrombosis), resulting in heart attack or stroke.[3] Beyond hemostasis and thrombosis, platelets are surprisingly multifunctional and are involved in the maintenance of vascular tone, inflammation, host defense, and tumor biology.[4]

Due to the high stakes involved in aberrant platelet activity, there is increasing emphasis on understanding platelet function and developing anti-platelet therapies.[3,4] The ability to accurately and precisely compute platelet concentration is crucial in both clinical and basic science research laboratories.

Overview of platelet counting

In 1953, Brecher et al developed a phase contrast microscopy method that enabled platelets to be manually discriminated from lysed RBCs and enumerated using a hemocytometer.[5] However, such manual counts are time consuming, subjective, and prone to error, with high inter-observer imprecision ranging from 10–25%, especially in samples with low counts (<50 x 10^9/L).[6]

Automated whole blood impedance counters resulted in dramatic increases in precision. Although this method is still widely used, impedance analysis is inherently limited by its inability to adequately discriminate platelets from other particles of similar size.[7,8,9] Further, accurate and reliable impedance counting depends upon accurate calibration of instruments. Until recently, despite its significant limitations, manual counting remained the only "gold standard" available in platelet counting to standardize automated analyzers and assess their accuracy.

The need remained for a reliable reference counting method capable of unambiguous detection and absolute enumeration of platelets, with accuracy across the entire range of platelet counts, to improve calibration of hematology analyzers and increase the accuracy of platelet counting at very low levels.[10]

Impact of flow cytometry

Flow cytometry offers a powerful and effective methodology for enumeration of platelets. In flow cytometry, particles or cells suspended in a hydrodynamically focused liquid stream pass through a beam of laser light (Figure 1). Optical detectors collect scattered laser light and fluorescence emissions, and electronics digitize these signals for computational analysis. The light scatter data provides basic information about the cells, such as relative size, shape, and surface features, and thus resolves platelets from noise, debris, and other cells such as RBCs. Labeling the cells with fluorescent dye-conjugated antibodies allows further resolution of platelets as well as quantification and exclusion of coincidence events (platelet/RBC or RBC/RBC complexes) that can obscure the platelet count. Thus, flow cytometry removes user-dependent counting errors and allows simultaneous measurement of multiple cellular parameters at the single-cell level.

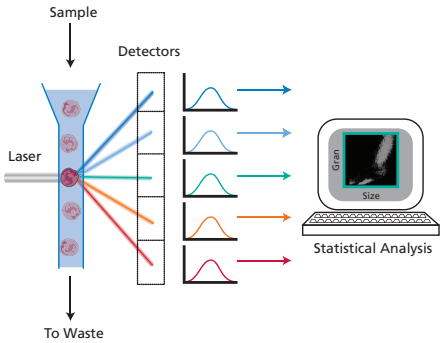

Figure 1. How flow cytometry works.

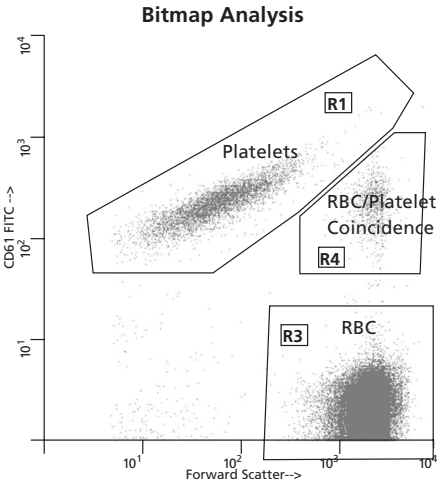

Figure 2. Bitmap analysis of platelets in whole blood samples using the RBC/Platelet ratio method.

Data from Harrison et al, 2001, reproduced courtesy of the authors and Cytometry Part A.

Platelet counting by the RBC/platelet ratio (IRM)

The International Council for Standardization in Haematology (ICSH) and the International Society for Laboratory Hematology (ISLH) now recommend an International Reference Method (IRM) for enumerating platelets on a flow cytometer.[11] In this method, the RBC/platelet ratio IRM, a flow cytometer is used to count fluorochrome-labeled platelets relative to RBCs, while an impedance counter is used to provide an accurate RBC count. The final platelet count is then calculated from the ratio and the RBC count.

For platelet counting using this method,[10,11] EDTA-anticoagulated whole blood samples are labeled with antibodies specific to distinct epitopes on the glycoprotein IIb/IIIa complex of resting and activated platelets (anti-CD41 and/or anti-CD61) to allow optimal resolution of platelets from noise and other cells. Samples are analyzed by flow cytometry at a final dilution of 1:1,000, which was found to eliminate the need to correct for coincident platelet/RBC or RBC/RBC events in most cases, at a flow rate of <4,000 events per second. Either quadrant or bitmap analysis is acceptable, but bitmap analysis is preferred (Figure 2).

To determine the RBC/platelet ratio, at least 1,000 platelet events and 50,000 RBC events are collected per sample. Events that are positive for both RBC scatter signal and platelet fluorescence are considered RBC-platelet coincidence events, which may require correction as explained by Harrison et al.[10] The platelet count is calculated from this ratio and the RBC concentration of the original blood specimen, as determined by impedance counting.

The RBC/platelet ratio IRM counting method is simple, rapid, reliable, and easy to implement in any laboratory with a flow cytometer. It demonstrates good correlation and superior precision to manual counting methods and is independent of pipetting and dilution artifacts. At least two studies[12,13] have already shown that automated analyzers tend to overestimate platelet counts when compared to the IRM platelet count.

Platelet counting by direct volume

Although the RBC/platelet IRM is reliable and accurate, the employment of both a flow cytometer and an impedance analyzer adds cost, complexity, and effort to platelet enumeration. Since all flow cytometers can count cells, it would be ideal if the flow cytometer alone could accurately report platelet concentration (per unit sample volume).

Digital flow cytometers with laminar-flow fluidics allow fast, phenotypic data collection (up to 10,000 events per second) on a wide range of cell types (submicron-sized bacteria through large mammalian cell lines). However, because these instruments cannot usually report sample volume directly, counting beads must be added to each sample to calculate cell concentrations. Since the bead concentration is known, the cell concentration can be calculated based on the ratio of beads to cells.

On the other hand, flow cytometers with syringe-driven fluidics can determine cell concentrations directly, without counting beads. However, they are often limited by lower data acquisition rates (<1,000 events per second), diminished fluorescence and light-scatter resolution, and a propensity for clogs in the flow cell.

The unique fluidics system of the BD Accuri C6 flow cytometer combines the advantages of these two types of instruments, allowing it both to count cells and accurately compute sample volume. This white paper reports the validation of the BD Accuri C6 to perform platelet counts in whole human peripheral blood specimens using the RBC/platelet ratio IRM and direct volume computation.

Technical considerations of the BD Accuri C6

Operating environment

The BD Accuri C6 flow cytometer (Figure 3) offers performance, simplicity, and affordability. Light, rugged, and small enough to fit on a benchtop, it connects to standard electrical circuitry and can even be placed in a laminar flow hood. Fixed optics and capillary sheath-flow fluidics make the BD Accuri C6 portable without the need for realignment.

Figure 3. The BD Accuri C6 personal flow cytometer.
The instrument weighs just 13.6 kg (30 lb). Exterior dimensions (H x W x D) are 27.9 x 54.6 x 41.9 cm (11 x 21.5 x 16.5 in.) with fluid tanks in place.

BD Accuri™ C6 software makes operation intuitive for novice and proficient users alike. Most new users become fluent with the software in less than 30 minutes, assisted only by a 3-page pictorial *Quick Start Guide*. Data files can be analyzed within the program, aided by special tools such as Zoom and VirtualGain™, or exported in FCS 3.0 format into FCS Express™, FlowJo™, and other flow cytometry analysis programs.

Routine instrument cleaning and priming are automated. Maintenance is easy, even for novice users, and requires no tools. The recommended sheath fluid is 0.22-µm filtered, deionized water. These attributes effectively expand flow cytometry beyond the core facility and onto the laboratory bench.

Optical and analytical modalities

The fully digital BD Accuri C6 features two lasers, two scatter detectors, and four fluorescence detectors. A state-of-the-art digital signal processing (DSP) system gives the BD Accuri C6 a dynamic range of six full decades. This means that it can finely resolve both faint and bright signals at once and analyze a wide span of variation in fluorescence in a single run. The instrument detects this broad dynamic range using standard factory detector settings, without the need for optimization or tuning.

If additional flexibility is needed, the optical configuration can be changed. The Selectable Lasers Module (Cat. No. 653126) allows reassignment of the standard laser-detector associations, and optional filters can modify the effective detector characteristics.

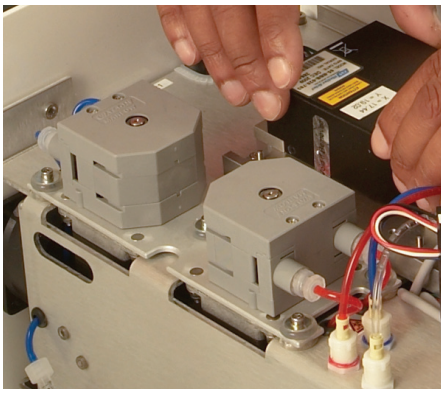

Figure 4. The BD Accuri C6 peristaltic pump system.

The unique laminar-flow fluidics system, driven by peristaltic pumps, combines the advantages of hydrodynamically focused cell sampling with the ability to report absolute cell counts for any identified population in a sample.

High-performance fluidics report volume automatically

The BD Accuri C6 flow cytometer has a unique laminar-flow fluidics system driven by peristaltic pumps (Figure 4). By monitoring the pressure in the Sample Introduction Probe (SIP), a microprocessor can determine the sample flow rate. This arrangement combines the advantages of hydrodynamically focused cell sampling (high data acquisition rates, good light-scatter and fluorescence resolution) with the ability to accurately compute sample volume and automatically report concentrations for any identified population in a sample.

This arrangement simplifies platelet analysis by automatically calculating cell concentrations during data collection ("direct volume counting"), eliminating the need to count manually or to standardize data with counting beads or RBC counts obtained using hematology analyzers. BD Accuri C6 software displays the volume (in µL) as data in the statistics tables and automatically calculates counts per µL for any gated population. Users can display the counts in a data table on the Statistics tab of the software.

The "push-pull" peristaltic pumps also enable independent regulation of both the sheath and sample flow rates. Users can quickly optimize the sample core diameter (adjustable from 5 to 40 µm) based on the anticipated size range of cells or particles within the sample. The design also allows easy removal of clogs (such as cell clumps) from the flow cell.

Validation of platelet counting on the BD Accuri C6

Study design

The BD Accuri C6 flow cytometer was used to obtain platelet counts for 144 EDTA anti-coagulated human whole blood samples using both the RBC/Platelet ratio IRM and the direct volume method. These two measurements on the same instrument were compared and correlated to each other, and to counts obtained using the IRM on a BD FACSCalibur system as a representative standard laboratory cytometer. The whole blood samples contained a broad range of platelet counts (1–700 x 10^9/L), allowing assessment of platelet counting in samples with very low counts, which prove problematic for many instruments.

Methods

Sample preparation

To allow comparison of platelet counts across methods of flow cytometric collection, samples from each blood specimen were diluted and stained within 24 hours of blood draw, according to the IRM sample preparation protocol.[11] Briefly, 20 µL of EDTA anti-coagulated whole blood (mixed by gentle inversion 6 times) was diluted 1:20 into 380 µL of PBS containing 0.1% bovine serum albumin (PBS-BSA buffer). Fifty microliters of diluted blood was combined with 2.5 µL of FITC-conjugated anti-CD61 (BD Biosciences, Cat. No. 348093) in 12 x 75-mm polystyrene tubes and diluted 1:50 to a total volume of 2,500 µL with PBS-BSA buffer, to achieve a final dilution of 1:1,000. Samples were incubated for 15 minutes at ambient temperature in the dark.

Sample analysis

Stained samples were gently inverted 6 times prior to analysis to ensure sample homogeneity, and then analyzed on both the BD Accuri C6 and the BD FACSCalibur until 50,000 RBCs and 1,000 platelets were collected, according to the protocol. Events positive for both RBC scatter signal and platelet fluorescence (FITC, FL1; ex:488, em:530/30) were considered coincidence events.

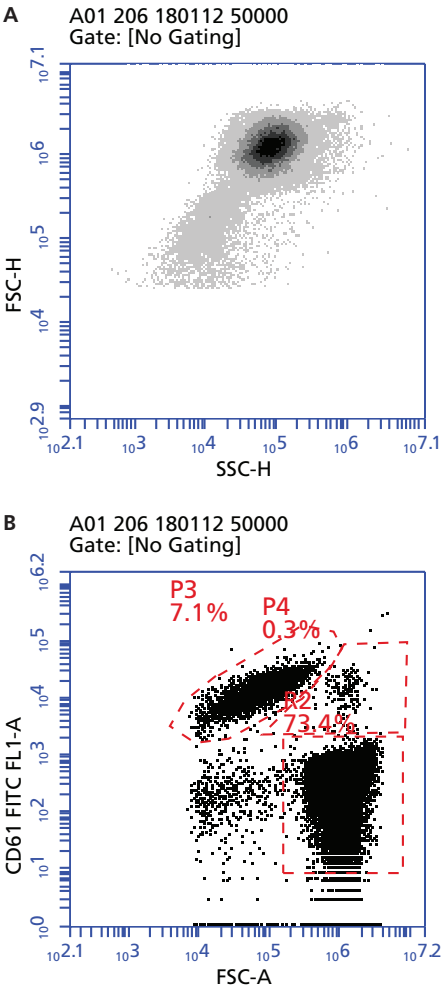

Figure 5. Optimized settings for platelet counting on the BD Accuri C6.

A. SSC-H vs FSC-H scatter plot of stained whole blood, collected using an FSC-H threshold of 25,000. **B.** *FSC-A vs CD61 FITC (FL-1) dot plot. Platelets ($FSC^{low}CD61^{+}$, P3) are clearly resolved from RBCs ($FSC^{high}CD61^{-}$, R2) and coincidence events with both RBC scatter signal and platelet fluorescence ($FSC^{high}CD61^{+}$, P4).*

To optimize direct volume counting on the BD Accuri C6, preventive maintenance and performance validation, including fluidics calibration, were performed prior to running experimental samples.[14] Samples were analyzed at Medium speed (< 3,000 events per second), using an FSC-H threshold of 25,000 to exclude debris (Figure 5A). Gates were drawn around RBC, platelet, and RBC/platelet coincident populations (Figure 5B).

Calculation of platelet concentration

On the BD Accuri C6, the platelet concentration (x 10^9/L) for each sample was determined in two ways: using the RBC/platelet ratio (IRM) and by direct volume counting. On the BD FACSCalibur, the platelet concentration was calculated using only the IRM.

Platelet concentrations determined using the IRM were calculated according to the following formula:[11]

$$\frac{\text{RBC count determined by impedance}}{R}\text{; where } R = \frac{\text{RBC events}}{\text{Platelet events}} \text{ obtained by flow cytometry}$$

Platelet concentrations were determined using direct volume counting on the BD Accuri C6 as follows:

$$\underset{\text{(including coincidence events)}}{\text{Gated platelet counts/µL}} \times \underset{\text{(final dilution of blood specimen)}}{\text{1,000}}$$

All platelet concentrations in this paper are reported as counts x 10^9/L.

Comparison of platelet counts

Platelet counts calculated using both methods on the BD Accuri C6 were compared to each other, and to counts obtained on the BD FACSCalibur. Linearity of data was assessed, and the strength of the relationships (correlation coefficients) was determined.

Perfect correlation can be achieved if the points lie along any straight line. However, perfect agreement is achieved only if two measurements give identical results for all individuals. Bland-Altman (mean-difference) analysis[15] was used to determine the level of agreement between the measurements performed on each blood specimen. To compare two measurements of each sample on a Bland-Altman plot, their mean is plotted on the x-axis and their difference on the y-axis. The mean of the two measurements is plotted because it is the best estimate of the true (unknown) value.

Bland-Altman analysis reports the bias, or average difference, between two measurement methods. Whether the bias associated with a measurement is acceptable depends on the accuracy required for interpretation of results. If the bias is low enough not to cause problems in data interpretation, the methods can be used interchangeably. For platelet counting, measurement bias has the greatest impact on samples with low counts. For example, counts of samples containing platelet counts in the normal and even low-to-normal range (>50 x 10^9/L) would be relatively unaffected by a bias of 3 or even 10 (x 10^9/L). However, the accuracy of counts in a sample containing only 10 x 10^9 platelets/L would be greatly affected by a bias of 3.

Influence of coincidence correction

When calculating platelet concentrations using the RBC/platelet ratio method, coincidence events are not counted, since their impact has been determined to be negligible.[10] However, when setting up new assays, assessment of coincidence correction is important to ensure that optimal conditions (such as dilution, flow rate, and acquisition rate) are achieved.[10] Figure 6 shows linear and Bland-Altman plots of data generated with (x-axis) and without (y-axis) coincidence correction on the BD Accuri C6 flow cytometer (left) and the BD FACSCalibur system (right). The perfect correlation of data on both instruments (R^2=1, Figure 6A) shows that, at the standard 1:1,000 dilution, coincidence events do not influence the RBC/platelet ratios in this study, as expected from previous work. Bland-Altman analysis (Figure 6B) also indicates a high agreement between the platelet counts with and without correction, as indicated by low bias values for measurements obtained on both instruments.

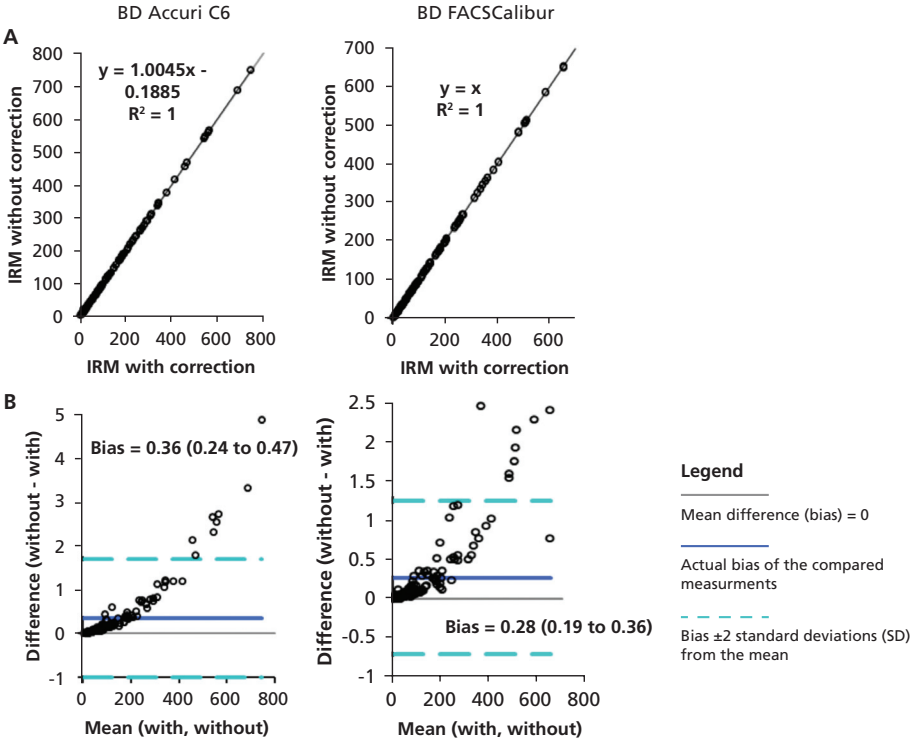

Figure 6. Coincidence correction is not required when using the RBC/platelet ratio (at 1:1,000 dilution) to calculate platelet concentrations.

A. Comparison of IRM platelet counts (x 10^9/L) in samples collected on the BD Accuri C6 (left) and on a BD FACSCalibur (right) with (x-axis) and without (y-axis) coincidence correction. ***B.*** *Bland-Altman (mean difference) analysis of IRM platelet counts, calculated with and without correction for coincidence events. The mean of the two counts for each sample is plotted on the x-axis, while their difference is plotted on the y-axis. The average difference between the two measurements is reported as the bias, with the 95% confidence interval shown in parentheses.*

However, similar analysis of direct volume counting on the BD Accuri C6 indicated that, at higher platelet concentrations, excluding coincidence events resulted in underestimation of platelet counts as indicated by a best-fit line with slope <1.0 (Figure 7A, slope = 0.97). Bland-Altman analysis more clearly demonstrates this difference: the negative difference between the two counts (without – with, y-axis) increases in magnitude as the counts rise (Figure 7B).

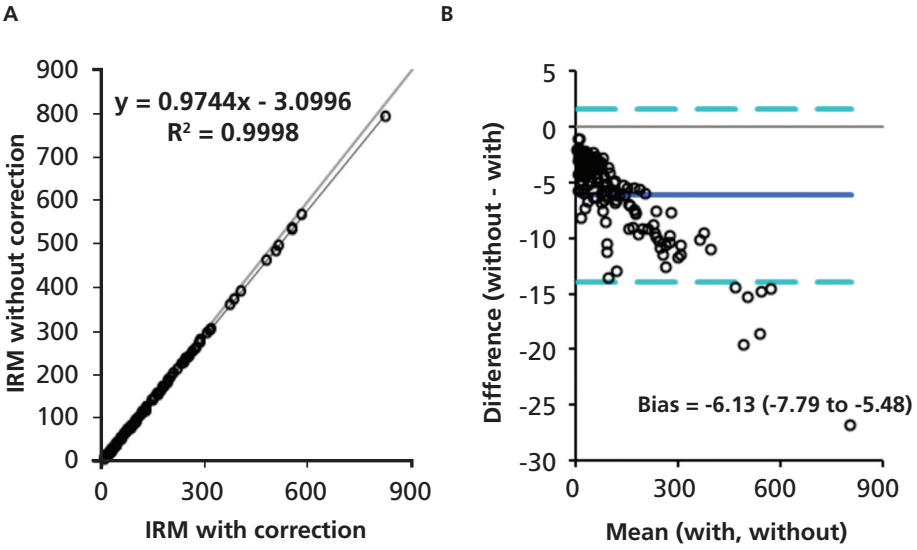

Figure 7. At higher concentrations, coincidence correction underestimates direct volume platelet counts on the BD Accuri C6.

A. Comparison of platelet counts (x 10^9/L) determined by direct volume computation on the BD Accuri C6 with (x-axis) and without (y-axis) coincidence correction. The best-fit line appears slightly below the theoretical diagonal of perfect agreement. ***B.*** *Bland-Altman analysis of platelet counts with and without coincidence correction shows negative bias at higher concentrations.*

In view of this data, all platelet counts reported in this paper include coincident platelet/RBC events.

Results

Platelet counting by the RBC/platelet ratio IRM

In Figure 8, platelet counts obtained using the RBC/platelet ratio IRM on the BD Accuri C6 were compared to counts using the same method on the BD FACSCalibur. This analysis determines whether the two instruments yield similar results when running matched samples using optimized instrument settings.

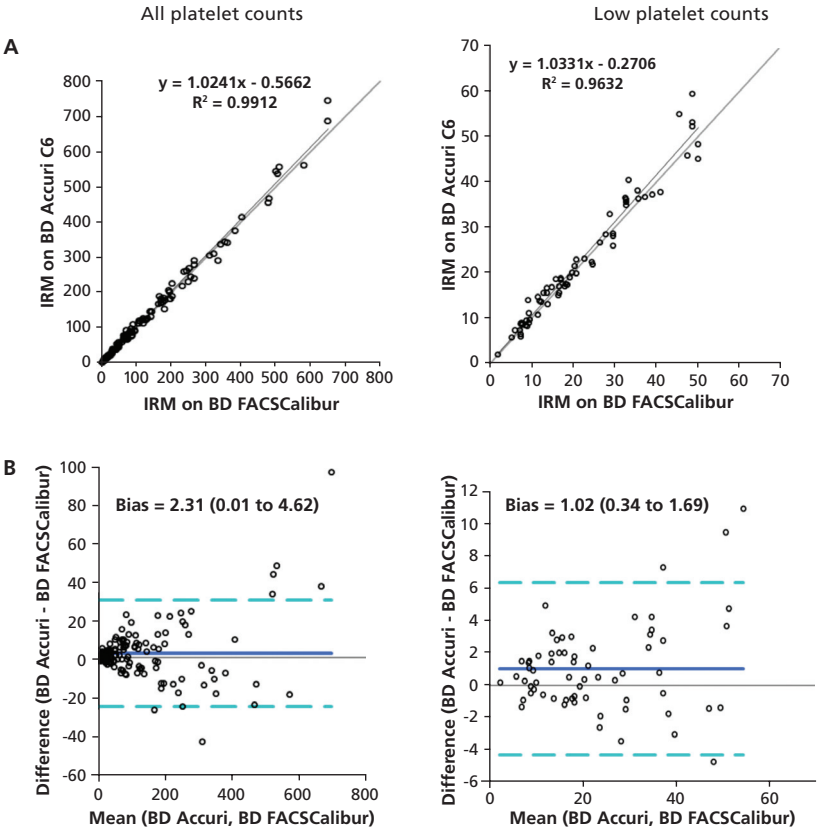

Figure 8. Platelet counts obtained by IRM on the BD Accuri C6 and BD FACSCalibur are interchangeable.

A. Comparison of platelet counts (x 10^9/L) obtained on the BD FACSCalibur (x-axis) and BD Accuri C6 (y-axis) using the RBC/platelet ratio IRM, for (left) all platelet counts and (right) low platelet counts (<50 x 10^9/L). ***B.*** *Bland-Altman analysis of the data in Figure 8A shows low bias between the two instruments for either range of concentration.*

The left panel of Figure 8A shows a high correlation across the entire range of platelet counts determined using the IRM on the BD Accuri C6 flow cytometer compared to the BD FACSCalibur flow cytometer (R^2 = 0.99). The right panel shows a segregated analysis of only the low-platelet (<50 x 10^9) subpopulation, which is especially problematic when counting manually. This analysis also demonstrated a high level of correlation between the counts obtained on the two instruments (R^2 = 0.96).

Counts obtained on the BD Accuri C6 tended to be slightly higher than counts obtained on the BD FACSCalibur, as indicated by slopes of 1.02 and 1.03 for the total and low-count populations, respectively. However, Bland-Altman analysis indicated low bias values for both populations (Figure 8B), indicating that these two instruments can be used interchangeably to enumerate platelets using the RBC/platelet ratio IRM.

Platelet counting by direct volume

Direct volume counting of platelets eliminates the need for additional measurement of RBC counts or the use of reference counting beads,[16] allowing more streamlined analysis and eliminating the cost of additional analyzers and reagents. Figure 9 shows comparisons of platelet counts between the direct volume method on the BD Accuri C6 and the RBC/platelet ratio IRM on the BD FACSCalibur.

When compared across the full range of platelet concentrations (left panels), the two methods show a high linear correlation (Figure 9A; $R^2 = 0.97$). Direct volume counts on the BD Accuri C6 tended to be slightly lower than IRM counts on the BD FACSCalibur (slope = 0.97). Notwithstanding, counts using these two different methods on two different instruments demonstrated a high level of agreement, resulting in a bias of –0.16 by Bland-Altman analysis (Figure 9B) and indicating that the two methods can be used interchangeably.

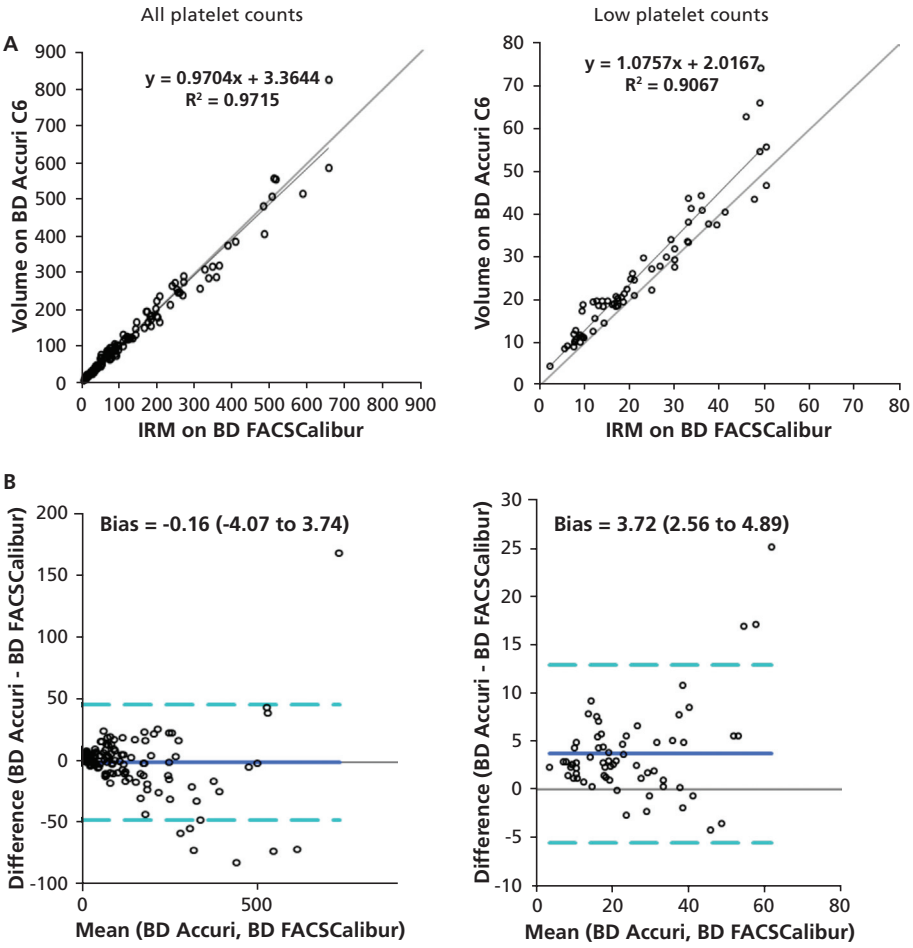

Figure 9. Comparison of direct volume platelet counts on the BD Accuri C6 to IRM counts on the BD FACSCalibur.

A. Correlation of counts (x 10^9/L) obtained using the RBC/platelet ratio IRM on the BD FACSCalibur (x-axis) and by direct volume on the BD Accuri C6 (y-axis) for (left) all platelet counts and (right) platelet counts < 50 x 10^9/L. B. Bland-Altman analysis shows (left) minimal bias between the two methods across the full range of platelet counts, and (right) modest positive bias for low platelet counts.

When only samples containing low platelet counts are compared (right panels), however, the correlation coefficient drops slightly (R^2 = 0.91), the slope increases to 1.08 (Figure 9A), and bias increases to 3.72 (Figure 9B). Thus, direct volume counting on the BD Accuri C6 flow cytometer might overestimate counts in samples that contain low platelet concentrations compared to the RBC/platelet ratio IRM on the BD FACSCalibur system. In a sample containing 50 x 10^9 platelets/L, the increased bias translates approximately to a 7% difference between the two methods, which is typically not problematic and compares favorably to other methods of measurement.

Comparison of IRM and direct volume methods on the BD Accuri C6

Lastly, both counting methods were compared on the BD Accuri C6 (Figure 10). Across all samples (left panels), platelet counts obtained by direct volume counting showed high correlation to counts obtained on the same instrument using the RBC/platelet ratio IRM (Figure 10A; R^2 = 0.99). The slope of 0.95 shows that counting by direct volume tended to slightly underestimate platelet counts compared to the RBC/platelet ratio. Bland-Altman analysis suggests that undercounting in samples containing high platelet counts (primarily >400 x 10^9/L) was primarily responsible for the lower slope and negative bias (Figure 10B, left). Nonetheless, the observed level of bias (–2.48) is acceptable and will minimally impact counts in the normal and even low-normal ranges.

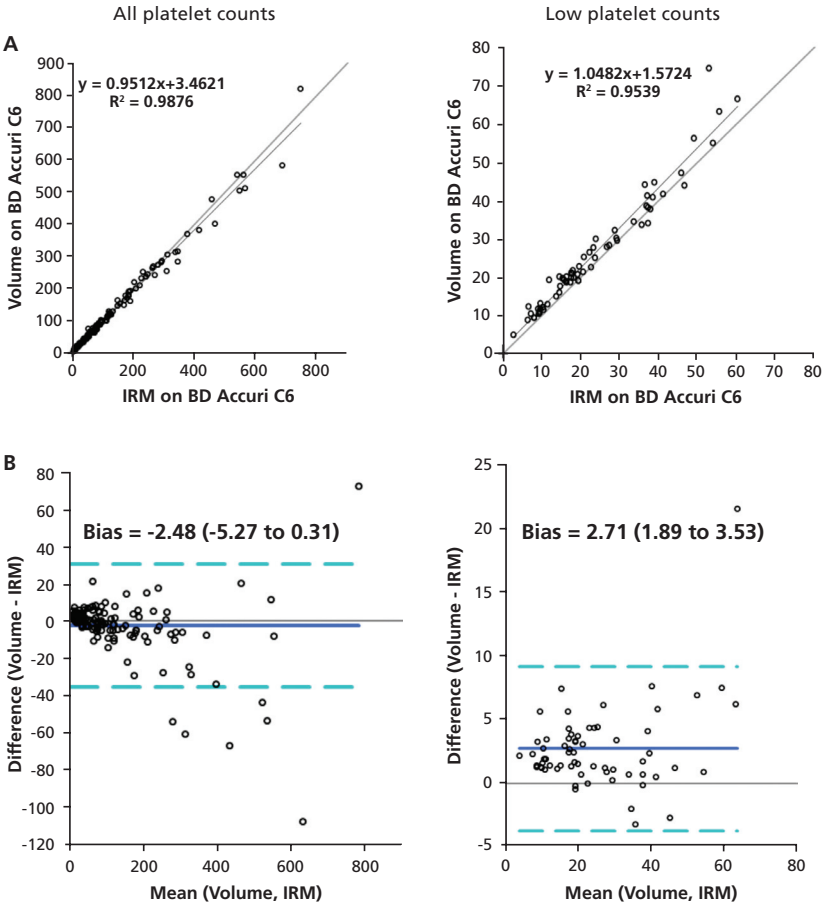

Figure 10. Comparison of direct volume and IRM platelet counts on the BD Accuri C6.

A. Correlation of counts (x 10^9/L) obtained using the RBC ratio IRM (x-axis) and by direct volume (y-axis) on the BD Accuri C6 for (left) all platelet counts and (right) platelet counts < 50 x 10^9/L. B. Bland-Altman analysis shows (left) modest negative bias between the two methods across the full range of platelet counts, and (right) modest positive bias for low platelet counts.

Samples with low platelet counts (Figure 10, right panels) again showed a tendency for direct volume counting to report higher counts than the IRM (R^2 = 0.96; slope = 1.05; bias 2.71). However, the correlation was higher, and the overcounting less pronounced, between the two methods on a single instrument than between the BD Accuri C6 and the BD FACSCalibur (Figure 9, right panels: R^2 = 0.91; slope = 1.08; bias = 3.72).

Reproducibility and linearity of data on the BD Accuri C6

The reproducibility of platelet counts using the RBC/platelet ratio IRM and direct volume method on the BD Accuri C6 was assessed in selected samples. To determine reproducibility, each sample was measured ten times, and the mean platelet count and CV determined. As shown in Table 1, CVs for the IRM on the BD Accuri C6 ranged from 3–5% and for direct volume counting from 4–10%. This level of reproducibility is comparable to the RBC/platelet IRM on the BD FACSCalibur (~5%)[12], as well as with CVs for impedance counters, which were recently reported in the 7.3–7.8% range when optimally calibrated.[16]

Table 1. Reproducibility of IRM and direct volume platelet counts on the BD Accuri C6.

Sample Number	RBC ratio	Volume
1	Mean = 44.3 CV = 4.9%	Mean = 53.0 CV = 4.1%
2	Mean = 243.7 CV = 3.1%	Mean = 231.8 CV = 10.4%
3	Mean = 486.3 CV = 2.96%	Mean = 504.2 CV = 4.4%

The linearity of platelet counts on the BD Accuri C6 was determined by serially diluting two samples containing high and low platelet counts, respectively (Figures 11A and 11B), to allow examination of counting at each extreme and across a wide dynamic range. Both dilution series resulted in a high linear correlation (R^2 ~ 0.99) across a wide range of concentrations and yielded lines with equations similar to those observed in analysis of the entire sample set (Figure 10A).

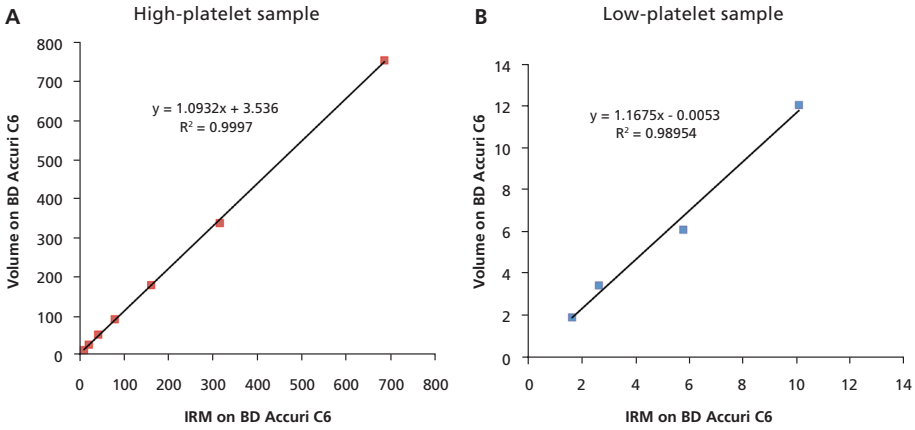

Figure 11. Linearity of platelet counts in selected samples after serial dilution.

Linearity of platelet counts obtained using the IRM (x-axis) and direct volume (y-axis) methods on the BD Accuri C6 after collection of serially diluted whole blood samples. ***A.*** *The platelet count was 700 x 10^9/L for this sample.* ***B.*** *The platelet count was 10 x 10^9/L for this sample.*

Summary and conclusions

The BD Accuri C6 flow cytometer is small, affordable, easy to use, and reliable. It can be used to count platelets using either the RBC/platelet ratio International Reference Method (IRM) or the direct volume method. This study indicates that, as expected, IRM counts obtained on the BD Accuri C6 are functionally interchangeable with IRM counts determined using a BD FACSCalibur flow cytometer. They correlate highly, show low bias, and have good reproducibility and linearity across the entire range of platelet counts encountered over 144 samples.

The direct volume method requires no additional measurement of RBC or reference bead counts, and offers the added benefits of increased speed and reduced cost and complexity. For platelet counts spanning the normal range, direct volume computation on the BD Accuri C6 was similarly found to be functionally interchangeable with the IRM on either instrument.

When analyzing samples with low platelet counts (<50 x 10^9/L), the direct volume method on the BD Accuri C6 showed a modest tendency to report higher counts than the IRM on either instrument. This too was expected, since the IRM calculation by nature corrects for such variables as pipetting error, whereas direct volume does not. For many studies, the higher counts will not affect results if either method is used consistently or when the differences fall within the range of measurement error. Researchers should be aware of this tendency and, if they suspect bias, may want to validate their method.

For the sake of comparison, the direct volume counts in this study were obtained using parameters optimized for the RBC/platelet ratio IRM (1:1,000 dilution, 50,000 RBCs and 1,000 platelets collected, etc). To obtain greatest accuracy, researchers using direct volume to count low-platelet samples should determine the optimal dilution and analysis parameters for their experimental samples.

In sum, direct volume counting of platelets on the BD Accuri C6 offers a simple, straightforward, new method for the accurate determination of platelet counts in research laboratories. This alternative is particularly attractive for laboratories that do not have access to a hematology analyzer, which is necessary to obtain accurate RBC counts for the IRM method.

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