A Rapid and Accurate Closed-Tube Immunoassay for Platelets on an Automated Hematology Analyzer

James E. Gill, PhD,1 Kenneth A. Davis, PhD,2 Willie J. Cowart, MS,1 Francisco U. Nepacena, BSMT,1 and Young-Ran Kim, PhD1

Key Words: Platelets; Thrombocytopenia; CD61; Platelet counting; Platelet transfusion; Hematology analyzer

Abstract

Accurate and precise platelet counts are important for patients with severe thrombocytopenia or who are receiving chemotherapy. We developed a novel flow cytometric analysis of platelets that may be particularly valuable for assessing the necessity for platelet transfusions. This ImmunoPlt (CD61) assay is based in part on CD61 monoclonal antibody labeling and has been automated and implemented on the CELL-DYN 4000 hematology analyzer. It is well suited for thrombocytopenic specimens, since it reduces interference by nonplatelet particles. It takes less than 5 minutes from closed-tube aspiration to report. Data for more than 350 thrombocytopenic specimens demonstrate that the ImmunoPlt (CD61) assay is more accurate than the optical scatter or the impedance count for specimens with platelet counts between 1 and 60 × 10^3/µL (1 and 60 × 10^9/L). The ImmunoPlt (CD61) assay is more precise than the optical scatter or the impedance count for specimens with platelet counts between 1 and 50 × 10^3/µL (1 and 50 × 10^9/L).

Accurate and precise platelet counts are important for patients being treated for thrombocytopenia or receiving chemotherapy. Platelet counts from hematology analyzers are based on the impedance or light-scatter technology and usually are valid at counts down to 50 × 10^3/µL (50 × 10^9/L). For blood with platelet counts less than 50 × 10^3/µL (50 × 10^9/L), accuracy of platelet counts may be compromised by the presence of nonplatelet particles (NPPs). These NPPs most often are RBC fragments but also may consist of WBC fragments, immune complexes, bacteria, and perhaps substances.1 In patients with severe thrombocytopenia, the number of NPPs may be equal to or greater than the number of true platelets. When the NPPs are counted as platelets, the consequences for the patient can be serious.2

Imprecision in platelet counting for specimens from patients with thrombocytopenia may occur with manual and automated methods. The manual method usually gives a reported platelet count in units of 10^9/L equal to the number of platelets actually counted. Thus, for a platelet count of 10 × 10^3/µL (10 × 10^9/L), only 10 platelets are seen and counted, so the Poisson error can be quite large. Proficiency testing results confirm that automated methods are more precise than manual methods for platelet counts less than 100 × 10^3/µL (100 × 10^9/L).4 However, a careful analysis of platelet counting precision by Hanseler et al5 indicates that for severely thrombocytopenic specimens, with platelet counts less than 30 × 10^3/µL (30 × 10^9/L), an automated system counting platelets with light-scatter technology may be less precise than the manual method. Also, Koh et al6 reported that about 70% of thrombocytopenic specimens, in which the platelet distribution width was abnormal, showed discrepant values between automated and
Manual counts. Thus, impedance or light-scatter automated platelet methods are no guarantee of superior precision or accuracy.

The use of fluorescent flow cytometric techniques for platelet identification and counting and the advantages these techniques offer have been described by several authors. Ault7 was the first author known to us to have described the use of immunologic markers to unequivocally identify platelets to improve platelet counting in patients with thrombocytopenia.

Valet8 developed a method wherein a sample of diluted blood was stained with the fluorescent dyes acridine orange and 3,3-dihexyloxacarbocyanine then analyzed by fluorescence and impedance. This method was said to provide absolute counts and relative proportions of erythrocytes, reticulocytes, platelets, lymphocytes, and granulocytes, as well as the functional state of various blood cell types.

Terstappen and Loken9 developed a method for blood and bone marrow differential counts wherein the specimens were stained with a mixture of 2 fluorescent dyes and the monoclonal antibody CD45 conjugated with phycoerythrin (PE). The fluorescent dyes were LDS751 and thiazole orange. After staining of the blood specimen and flow cytometric analysis, platelets were characterized by their relatively low forward and orthogonal light scattering and by their uptake of LDS751. This method could provide relative but not absolute counts.

Dickerhoff and von Ruecker10 described the use of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody for platelet counting. They used a mixture of CD42b and CD41, both conjugated with FITC. To overcome the inability of their flow cytometer to measure sample volume with sufficient accuracy, they added a known number of FITC-fluorescent latex particles to an accurately known volume of blood. They then used a fluorescence threshold to count FITC-stained platelets and FITC beads selectively and determined the platelet count by multiplying the FITC platelet/FITC bead ratio with the known FITC bead concentration.

We developed a novel technology, combining the fluid metering required for absolute counts and CD61 monoclonal antibody labeling of platelets, to fully automate an immunoassay for platelets. The reagent includes CD61-FITC in a lyophilized protective matrix that is stable for 1 year at room temperature. This reagent enabled us to automate the method on a hematology analyzer and shorten the incubation time to just 1 minute. The method lends itself to the rigorous standardization and quality control procedures usually associated with hematology analyzers. Thus the CELL-DYN ImmunoPlt (CD61) assay (Abbott Diagnostics Division, Santa Clara, CA) for severely thrombocytopenic specimens can become a rapid routine procedure in any hematpathology laboratory. This assay provides a substantial advance in safety, efficiency, and quality control.

**Materials and Methods**

**CELL-DYN ImmunoPlt (CD61) Reagent**

The fully automated CELL-DYN ImmunoPlt (CD61) assay uses an FITC-conjugated CD61 monoclonal antibody (clone RUU-PL 7F12, BD Biosciences, San Jose, CA) in a lyophilized pellet. The pellet is held at the bottom of a 12 × 75-mm K-Resin tube by a small stainless steel retainer. The tube is capped with a HEMOGARD closure (Becton Dickinson, Franklin Lakes, NJ) and sealed in a moisture- and light-proof pouch. On addition of a blood specimen to the tube, the pellet immediately dissolves, releasing the CD61-FITC antibody. This reagent tube was codeveloped by BD Biosciences and Abbott Laboratories Diagnostics Division and is provided to Abbott Laboratories Diagnostics Division exclusively by BD Biosciences.

**Liquid Monoclonal Antibodies**

For preliminary studies, liquid monoclonal antibody conjugated with FITC or PE was obtained from various commercial sources, including the following: CD61-FITC from BD Biosciences; glycophorin A–FITC and CD41-PE from DAKO, Carpinteria, CA; and glycophorin A–FITC, CD41-PE, and CD42b-PE from Immunotech, Westbrook, ME.

**Blood Sources and Collection**

Blood specimens with normal hemograms, including platelet counts of 150 × 10³/µL or more (150 × 10⁹/L or more), were obtained from in-house donors who had given informed consent. Blood specimens with abnormal counts or possible interferences with the platelet count were obtained from local hospitals after completion of CBC testing. Specimens were collected into tubes with tripotassium EDTA as the anticoagulant, unless otherwise noted. Specimens from local hospitals were accompanied with a CBC report, including a platelet count but without diagnosis or patient identification.

**CELL-DYN 4000 Analysis**

For preliminary studies on prototype instruments, 40 µL of antplatelet monoclonal antibody and 40 µL of glycophorin A were combined with 20 µL of blood in a 12 × 75-mm tube and incubated for 4 to 15 minutes. Then, 2 mL of CELL-DYN 4000 Diluent/Sheath (Abbott) was added with gentle mixing; 0.5 mL of this mix then was combined with 3.5 mL of Diluent/Sheath for a final 1:840
dilution of blood. Approximately 1.0 mL of this dilution was analyzed by flow cytometry on the prototype instruments. CELL-DYN 4000 analyzers include an argon ion laser and are capable of detecting green fluorescence from FITC, orange fluorescence from PE, and red fluorescence from propidium iodide. The instruments also can detect axial light loss, intermediate angle scatter (IAS), and polarized side scatter (PSS) from cells as they pass through the laser beam.

In contrast with the prototype instruments, the current CELL-DYN 4000 fully automates sample preparation and analysis. A precision piston pump controls the aspiration of blood from the specimen tube and deposition of blood plus Diluent/Sheath into the single-use reagent tube. The contents of this container are mixed by gentle rocking at room temperature for approximately 1 minute. The system adds more Diluent/Sheath, mixes, aspires, and transports the diluted blood-antibody mixture to the optical flow cell for flow cytometric analysis. When performing ImmunoPlt (CD61) analysis, the prototype collected 4 parameters: IAS, PSS, green fluorescence, and orange fluorescence. The current CELL-DYN 4000 performs ImmunoPlt (CD61) analysis with IAS, PSS, and green fluorescence.

Before sample analysis, the CELL-DYN 4000 performance was tested and, if necessary, restandardized by running a series of latex particles, fixed cells, and commercial control materials and verifying that all measured parameters associated with these materials were within the specified ranges.

Data Collection and Analysis

List mode data collected during the prototype phase of the CELL-DYN 4000 development were reformatted and analyzed with Data Desk software (Data Description Inc, Ithaca, NY). Platelets were identified as green fluorescence–positive events with IAS values above threshold. Plots, linear least squares curve fits, and $R^2$ values for platelet counts were generated with DeltaGraph software (DeltaPoint Inc, Monterey, CA). The correlation between platelet counts based on different monoclonal antibodies or different techniques was analyzed with Excel (Microsoft, Redmond, WA).

Phase Microscopy Counts

Our phase microscopy counting procedure is based on the procedure described by Brecher and Cronkite. Two modifications, a change from 1:100 dilution to 1:25 dilution of blood and the use of 2 Unopette Microcollection reservoirs (Becton Dickinson) and 2 hemocytometers, were made to improve counting statistics for thrombocytopenic specimens. The charged hemocytometers were placed on the microscope stage, and platelets in the 4 large corner squares and the large center square of both chambers were counted. The total number of platelets counted in these 10 squares, multiplied by the dilution and divided by 1,000, gave the platelet concentration in units of $10^9/L$. Thus, for a platelet count of $10 \times 10^9/L$ ($10 \times 10^3/\mu L$ in traditional units), 400 platelets are seen and counted. This modified phase microscopy count provides improved precision, but at a high cost.

Blood Smear Preparation and Examination

Blood smears for platelet estimates were prepared with EDTA-anticoagulated blood no later than 8 hours after collection. Slides were prepared using the slide-to-slide method in which a small drop of blood is placed near the frosted end of a clean glass slide, and a second slide is used as a spreader. The smear was allowed to air dry and then was stained with Wright-Giemsa.

For correlation with the ImmunoPlt (CD61) assay, we examined smears with 3 known clinical manifestations: large platelets, giant platelets, and platelet clumps. Usually, examinations of smears for abnormal platelets use descriptive terms such as rare, occasional, few, moderate, or many. To quantify our findings for algorithm and flagging development, we developed a numeric model for classifying the smears 

<table>
<thead>
<tr>
<th>Frequency of Occurrence</th>
<th>No. of Large or Giant Platelets or Platelet Clumps†</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare or occasional</td>
<td>1</td>
<td>1+</td>
</tr>
<tr>
<td>Few</td>
<td>2-3</td>
<td>2+</td>
</tr>
<tr>
<td>Moderate</td>
<td>4-5</td>
<td>3+</td>
</tr>
<tr>
<td>Many</td>
<td>6 or more</td>
<td>4+</td>
</tr>
</tbody>
</table>

$^*$ Large platelets have diameters in the range of 4.0 to 6.0 µm; giant platelets have diameters of 6.0 µm or more.  
$^†$ Per 8 high-power fields in an area where the RBC’s touch but do not overlap.

Identifying and Resolving Platelets From NPPs

In the course of developing the optical platelet count on the CELL-DYN 4000, we occasionally noticed nearby clusters...
lying below or above the platelet cluster, when data was displayed in an IAS-PSS scatterplot. **Figure 1** shows 2 examples of early data showing the platelet clusters enclosed within 2 parallel lines. The data in Figure 1A are derived from normal blood and Figure 1B from a blood sample with a low platelet count. In Figure 1B, there are 2 additional clusters: 1 (no. 1) below and to the right of the platelet cluster and the other (no. 2) above and to the left.

To identify the events that formed these clusters, we labeled selected specimens with pairs of antibodies and analyzed these labeled specimens on a prototype CELL-DYN 4000. A portion of the sample represented in Figure 1B was labeled with a mixture of glycophorin A–FITC and CD41-PE and analyzed on this prototype (see the “Materials and Methods” section for details). **Figure 2A** shows a scatterplot of (green fluorescence and orange fluorescence) data from this analysis. The CD41+ and glycophorin A–positive clusters are well resolved from one another. And, about 7% of the events are in the region denoted negative.

When the events within the CD41+ gate are displayed in an IAS-PSS scatterplot **Figure 2B**, they form a cluster closely resembling the platelet cluster shown in Figure 1B. When the events within the glycophorin A–positive gate are displayed in an IAS-PSS scatterplot **Figure 2C**, they form a cluster resembling cluster 1 in Figure 1B and extending into the space occupied by the platelet cluster. This resemblance suggests that the events in cluster 1 are glycophorin A–positive and, therefore, presumably are RBC fragments. When the events within the negative gate are displayed in an IAS-PSS scatterplot, the events are diffuse but with some concentration in the region of cluster 2 (Figure 1B). Thus, the majority of events in cluster 2 are negative for both CD41 and glycophorin A. A minority of the events in cluster 2 are positive for glycophorin A and, thus, probably are RBC fragments. **Figure 2D** is an IAS-PSS scatterplot showing the overlap of CD41+ and glycophorin A–positive events identified in Figure 2A.

**Comparing CD41 or CD42b With CD61 for Platelet Counting**

Our early studies with CD41 or CD42b confirmed that it was possible to identify and count platelets and to resolve the platelets from NPPs (Figures 2A-C). We ran a comparison study to determine whether the platelet counts obtained with CD61 would match the counts obtained with CD41 or CD42b. Aliquots of 48 specimens were labeled with CD61 and with either CD41 or CD42b. The immunoassay platelet counts for these aliquots were obtained on the prototype CELL-DYN 4000. The immunoassay platelet counts obtained using CD61-FITC were compared with the counts obtained using CD41-PE or CD42b-PE. The result of this comparison is shown in **Figure 3**. The correlation is strong, with an $R^2$ value more than 0.99. The linear regression analysis gives an intercept that is not significantly different from zero and a slope that is not significantly different from 1.0. We concluded from these data that the immunoassay
platelet count obtained using CD61-FITC is equivalent to the count obtained using CD41-PE or CD42b-PE.

Comparing Immunoassay Platelet Counts With Phase Microscopy Counting

The accuracy of the CD61 immunoassay platelet count was determined by comparing this count to a phase microscopy count of the same specimens. At the same time, CELL-DYN 4000 optical and impedance platelet counts for these specimens also were obtained. Abnormal specimens with a platelet count of $70 \times 10^3/\muL$ or less ($70 \times 10^9/L$ or less), as reported by the hospital providing the specimen, were chosen for this comparison. The phase microscopy counting method was modified as described in the “Materials and Methods” section to improve the precision of counts from specimens with low platelet counts. The correlation of each automated method with the manual phase microscopy method is shown in Figure 4. The equation for the least squares linear fit and the correlation coefficient, $R^2$, are included. It is apparent from the plots that the correlation is
strongest between the CD61 count and the phase microscopy count and weakest between the impedance count and the phase microscopy count. The $R^2$ values for the different correlations confirm this impression.

**Stability of the CD61 Reagent**

Three lots of the CD61 reagent were tested for stability. Baseline data were collected within 1 week of manufacture. Tests were performed monthly, from 1 month after manufacture through 13 months after manufacture. At the beginning of the stability test, pouches of reagent were placed in cabinets with humidity controlled to 46% ± 15% and temperature controlled to 22.5°C ± 7.5°C. Pouches of reagent were kept in these cabinets until needed for stability testing. The tests consisted of analyzing normal blood specimens, diluted to give nominal platelet counts of 200, 100, and $50 \times 10^3/\mu$L. Each level was tested in triplicate with reagent drawn from all 3 lots. Throughout the 13 months of testing, all lots of reagent gave data that passed the test in every instance.

**Precision of Immunoassay Platelet Counting**

In designing experiments to determine the precision of immunoassay platelet counting, we were led to the paired differences method\textsuperscript{11} by 3 considerations: (1) Many of the clinical specimens with low platelet counts that we obtained consisted of 1 mL or less of blood, which was insufficient to allow for multiple replicate measurements. (2) The process of the immunoassay platelet count, if repeated more than several times on the same specimen, may cause subtle changes, such as platelet activation, that could influence the platelet count. Thus, the specimen itself could change during the course of 31 replicate measurements. (3) The paired differences method lends itself to demonstrating the precision over a range of platelet counts.

To determine precision, the platelet count range was divided into 2 regions: (1) $50 \times 10^3/\mu$L or less ($50 \times 10^9/L$ or less) and (2) more than $50 \times 10^3/\mu$L ($>50 \times 10^9/L$). Specimens were run in duplicate on the CELL-DYN 4000 and assigned to 1 of the regions based on the immunoassay platelet count. For each region, the coefficient of variation percentages at 95% confidence were calculated for the CD61, optical, and impedance platelet counts. They demonstrate that the immunoassay platelet count is more precise than the optical or impedance count in the region $50 \times 10^3/\mu$L or less ($50 \times 10^9/L$ or less).

**Linearity of Immunoassay Platelet Counting**

To test the linearity of the CD61 platelet count as performed on the CELL-DYN 4000, normal specimens with a reported platelet count of approximately $400 \times 10^3/\mu$L ($400 \times 10^9/L$) are diluted serially, with Diluent/Sheath, giving dilutions with nominal counts of 400, 200, 100, 50, 25, and $12.5 \times 10^3/\mu$L ($400, 200, 100, 50, 25, and 12.5 \times 10^9/L$). Each dilution is then run in duplicate. The CD61 platelet counts at each level are averaged; these average values are plotted against the nominal count, and the least squares linear regressions are calculated.

**Table 2**

<table>
<thead>
<tr>
<th>Method</th>
<th>50 $\times 10^3/\mu$L or Less (N)</th>
<th>&gt;50 $\times 10^3/\mu$L (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmunoPlt (CD61) assay</td>
<td>2.89 (65)</td>
<td>2.21 (74)</td>
</tr>
<tr>
<td>Optical</td>
<td>6.53 (65)</td>
<td>3.78 (70)</td>
</tr>
<tr>
<td>Impedance*</td>
<td>8.49 (46)</td>
<td>4.36 (82)</td>
</tr>
</tbody>
</table>

* Impedance platelet counts of less than $20 \times 10^3/\mu$L ($<20 \times 10^9/L$) are not reported by the CELL-DYN 4000 (Abbott Diagnostics Division, Santa Clara, CA) and are not included in the calculation of the coefficient of variation percentage for impedance.
Analysis of 189 blood specimens with reported platelet counts of less than $50 \times 10^9/\mu L$ ($50 \times 10^9/L$) were analyzed on a CELL-DYN 4000 (Abbott Diagnostics Division, Santa Clara, CA) to obtain the impedance, optical, and ImmunoPlt (CD61) counts. Portions of these specimens also were subjected to a phase microscopy platelet count as described in the “Materials and Methods” section. A, Immunoassay platelet count (CD61) vs phase microscopy platelet count; $y = 1.0225x - 0.4877$; $R^2 = 0.9717$. B, Optical platelet count vs phase microscopy platelet count; $y = 0.9514x + 1.544$; $R^2 = 0.9424$. C, Impedance platelet count vs phase microscopy platelet count; $y = 0.9041x + 6.8644$; $R^2 = 0.8533$.

As a test of system linearity, a blood specimen was diluted serially, each dilution was analyzed, and the residual percentages were calculated at each nominal platelet count. (The expression for the residual percentage is shown in the inset. $Y_i$ is the reported count, $N_i$ is the nominal count, $a$ is the slope, and $b$ is the intercept of the linear regression $Y$ vs $N$.) When the residual percentages are plotted against the nominal platelet count, the ImmunoPlt (CD61) assay values fall within the limits set by the system specifications. The limits shown are at $\pm 4\%$ or $\pm 5 \times 10^9/\mu L$ ($5 \times 10^9/L$), whichever is greater. The plot shows the result from 1 such experiment. The results have been reproduced on several instruments at different times and in several laboratories. Squares indicate residual percentages (CD61); circles, upper limit; and triangles, lower limit.
Identification of Large or Giant Platelets and Platelet Clumps

Platelet counting methods that identify platelets based on impedance or light scatter may identify very large and giant platelets as RBCs and thus exclude them from the platelet count. The ImmunoPlt (CD61) assay provides a scatterplot that includes large and giant platelets; these events are not excluded from the platelet count. Figure 6A shows an IAS-CD61 scatterplot obtained from a specimen graded as 2+ giant platelets with RBC fragments. (Note that the platelet cluster in this scatterplot is shifted up in IAS compared with the normal specimen platelet cluster shown in Figure 7B.) It seems that the platelet cluster in Figure 6A extends into the RBC-platelet coincidence cluster. Figure 6B shows the IAS-PSS scatterplot obtained from the same specimen. In this scatterplot, the platelet cluster extends upward in IAS underneath the RBC-platelet coincidence cluster. The platelet events that fall underneath the RBC-platelet cluster are associated with giant platelets that might be excluded from a platelet count based on impedance or light-scatter technologies. Note that giant platelets do not form a discrete population but are the largest members of a unimodal size or light-scatter distribution.

Platelet clumping is a common cause of falsely low platelet counts. These clumps are usually large enough to be excluded from the platelet count based on impedance or light-scatter methods. This exclusion occurs because the clumps give impedance values or light-scatter values in the range of RBCs or even WBCs. An advantage of the immunoassay platelet count is that these clumps are made visible in the IAS-CD61 scatterplot. Thus, the presence can be reported as a flag or alert by the CELL-DYN 4000.

The blood of certain persons (sometimes referred to as “EDTA clumpers”) contains platelets that tend to clump in the presence of EDTA. (EDTA is the most widely used anticoagulant.) This clumping can be avoided by drawing the blood into tubes containing an alternative anticoagulant, such as sodium citrate. Figure 7A and Figure 7B show IAS-CD61 scatterplots for a blood specimen drawn into EDTA or sodium citrate, respectively. Figure 7A shows an extended cluster of events above and to the right of the platelet cluster. In contrast, Figure 7B shows few events in this region. Therefore, we believe the events in this region represent platelet clumps.

Limitations and Interferences With Immunoassay Platelet Counting

Persons with Glanzmann thrombasthenia have platelets that have abnormalities in or lack the CD61 antigen. Thus the immunoassay platelet count method is inappropriate for blood specimens from these persons. These specimens will be flagged for review as the data will indicate a failure to label the platelets. Glanzmann thrombasthenia is rare. It is conceivable that other conditions exist in which affected persons would lack the CD61 antigen, but we have not seen cases of such a condition.

Platelet aggregation can lead to underreporting of the true platelet count when the platelet count is performed by the immunoassay platelet counting method (or by the impedance or light-scatter methods). With the immunoassay platelet counting method, each platelet aggregate is seen as a single event. This is true when the event consists of several platelets (or more) stuck together or stuck to a WBC, a condition known as platelet satellitism. With the immunoassay platelet counting method, in contrast with the impedance or light-scatter methods, the presence of aggregates is noted in the scatterplot, and a platelet aggregate alert is provided.

As with any assay using mouse antibodies, the possibility exists for interference by human antimouse antibodies in the specimen.

Discussion

More than 7 million units of platelets are transfused each year in the United States alone. The majority of platelet transfusions are administered to prevent bleeding in patients receiving chemotherapy. Bone marrow and stem cell transplants are other clinical situations usually requiring platelet transfusions. The primary diagnostic tool in determining when to transfuse a patient with platelets is the patient’s platelet count. Usually these patients receive a platelet transfusion when their platelet count is less than 20 \( \times 10^3/\mu L \) (20 to 109/L).

While transfusions decrease bleeding, there are associated risks and costs. The physical risks resulting from platelet transfusions include the transmission of infectious diseases, alloimmunization, immunosuppression, bacterial contamination, and febrile reactions. A number of groups have issued practical guidelines to minimize the incidence of adverse transfusion reactions and to decrease costs. To reduce these risks and costs, clinicians are reviewing the possibility of reducing the platelet transfusion trigger point from 20 to 10 \( \times 10^3/\mu L \) (20 to 109/L). The results of 2 studies suggest that this reduction would reduce the number of platelet transfusions by 20% to 40%, without a negative effect on patient care.
The availability of accurate, rapid, and precise platelet counts for specimens from patients undergoing bone marrow or stem cell transplants and chemotherapy is essential if patients are to benefit from this change to a lower trigger point. The ImmunoPlt (CD61) assay offers clear advantages over other methods for platelet counting of specimens from patients with thrombocytopenia. It is a completely automated assay, offering greater safety and convenience than traditional flow cytometric methods. It lends itself to the rigorous standardization and quality control procedures usually associated

**Figure 6** These scatterplots were obtained by analyzing a specimen containing “giant” platelets. The intermediate angle scatter (IAS)-CD61 scatterplot contains 2 green clusters of events and 1 white cluster of events. Events in the green diagonal cluster represent platelets; events in the green vertical cluster represent coincidence events, the simultaneous passage of a platelet and an RBC through the optical flow cell. Both types of events are counted as platelets. The events in the white cluster are nonplatelet particles. A, The green diagonal platelet cluster is shifted upward in IAS compared with the position of the platelet cluster in scatterplots obtained with normal blood (see Figure 7B for an example). B, The IAS-polarized side scatter shows the upper end of the platelet cluster has the same location in IAS as the RBC-platelet events. FL1, green fluorescence.

**Figure 7** A, The occurrence of platelet clumping can be seen in the intermediate angle scatter (IAS)-CD61 scatterplot. The clump events form a streak that extends up and to the right of the primary platelet cluster. The clumps formed when the blood was drawn into a collection tube containing EDTA. B, In contrast are the IAS-CD61 scatterplots obtained from the same patient’s blood but drawn into a tube containing sodium citrate as the anticoagulant. Few events could be considered clumps in this scatterplot, which is almost the same as those obtained with normal blood specimens. FL1, green fluorescence.
with hematology analysis. It is much less sensitive to interferences from NPPs than optical and impedance methods. It is free from interference between microcytes and large or giant platelets. It is also more accurate and more precise than other automated methods. And it is cost-effective compared with manual methods when manual methods are modified to accommodate severe thrombocytopenia.

From the 1Research & Development Department, Abbott Diagnostics Division, Santa Clara, CA, and 2BD Biosciences, San Jose, CA.

Address reprint requests to Dr Kim: Abbott Diagnostics Division, 5440 Patrick Henry Dr, Santa Clara, CA 95054.

Acknowledgment: We thank the clinicians who advised and encouraged us during the development of the ImmunoPlt (CD61) assay.

References