Detection of Platelet Clumps on Peripheral Blood Smears by CellaVision DM96 System and Microscopic Review

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ABSTRACT

Objective: To determine and optimize the sensitivity of the CellaVision DM96 automated image-analysis system in detecting platelet (PLT) clumps on blood smears and to assess the reliability of the traditional laboratory practice of examining only the feather edge of the smear for PLT clumps.

Methods: We processed 102 blood smears that revealed PLT clumps on microscopic review, using the CellaVision DM96, and reviewed the results for the ability of the analyzer to detect these clumps. We obtained the data regarding relative distribution of PLT clumps on different parts of the blood smear (feather edge, lateral edges, and readable area) from our microscopic-review observations.

Results: The sensitivity of the Cellavision DM96 in detecting PLT clumps was between 40.4% and 82.8%, depending on the number of screens reviewed for this variable. Via microscopic review of the smears, the PLT-clump detection rate increased from 85.3%, obtained by examining only the feather edge, to 99.0%, obtained by examining the feather edge plus the readable area.

Conclusion: The sensitivity of the DM96 for detecting PLT clumps can be maximized to 82.8% by reviewing the entire white blood cell screen and the entire PLT screen. Microscopic review of the blood smears yielded a PLT-clump detection rate of 99.0% when we examined the feather edge and the readable area of the smear.

Keywords: CellaVision DM96; DM96; platelet clumps; platelet scans; platelet estimates; blood smears

The CellaVision DM96 (DM96) is an automated image-analysis system (CellaVision AB, Lund, Sweden) designed to perform 3 functions associated with the differential leukocyte counts on peripheral blood smears. It identifies and preclassifies nucleated cells for the differential, precharacterizes aspects of the red blood cell (RBC) morphology, and estimates platelet (PLT) counts from the peripheral blood smear stained with a Romanowsky stain (Wright or Wright-Giemsa). These 3 sets of results are displayed on the white blood cell (WBC) screen, RBC screen, and PLT screen, respectively, for review by a laboratory technologist. The overall reliability of each of these functions of the DM96 has been assessed by a number of investigators, who found the functions to be acceptable for clinical use. However, to our knowledge, no published studies have assessed the reliability of this system in specifically detecting the presence of PLT clumps on peripheral blood smears. The presence of PLT clumps can render a numerical PLT estimate from the peripheral blood smear unreliable and thereby unreportable. For this reason, the system should be able to detect and identify PLT clumps with a sensitivity of nearly 100%. PLT clumps on the blood smear identified by the DM96 are displayed as PLT aggregates below the images of all WBCs on the lower part of the WBC display.

We sought to determine and optimize the sensitivity of the DM96 for detecting PLT clumps on the peripheral blood smears. A secondary goal was to assess the relative distribution of PLT clumps over different parts of the blood smear and to determine whether traditional laboratory practices, which examine only the feather edge of the stained blood smears for PLT clumps, are adequate for detecting the presence of this variable.
Materials and Methods

In our clinical laboratory at Thomas Jefferson University Hospital, Philadelphia, Pennsylvania, during an 18-month period, we collected a total of 102 blood smears that revealed significant PLT clumping on microscopic review; we processed those smears using the DM96 to determine the complete differential leukocyte count. All smears were from ethylenediaminetetraacetate (EDTA)-anticoagulated venous blood specimens tested within 4 hours of collection. The 102 blood specimens were collected from 79 patients, whose ages ranged from 0 days (newborn) to 82 years. We prepared most of the smears (~85.0%) using an automated system (SP1000i or SP100 Automated Hematology Slide Preparation Unit, Sysmex Corporation, Kobe, Japan) and manually prepared the rest (~15.0%). The latter represented most of the specimens collected in EDTA-containing microtainers via capillary puncture. We stained all smears with Wright stain, using the staining function of the same instruments, and examined the resulting smears microscopically at ×100, ×500, and ×1000 magnification.

On microscopic review of the blood smears, we considered PLT clumping to be significant whenever we judged the automated PLT count to be unreliable and thereby unreportable. The data collected from the microscopic review of each smear for the study included the relative distribution of PLT clumps on different parts of the smear (feather edge, 2 lateral edges, and readable area); the presence of fibrin strands with or without PLT clumps; and the report of the PLT estimate as being normal with clumps, decreased with clumps, or increased with clumps, per the judgment of the technologist. The data collected from the DM96 for each smear included whether the DM96 detected, identified, and displayed PLT clumps as PLT aggregates on the WBC screen; whether the reviewer had observed PLT clumps while reviewing the images on the WBC, RBC, or PLT display, and whether the reviewer observed fibrin strands on any of the displays. Our microscopic review generated a complete data set on all 102 smears that we examined; also, we were able to compile a complete data set from the DM96 for 99 of the 102 smears included in the study. The remaining 3 smears did not yield a complete data set because they were processed through the DM96 only for the PLT scan, rather than for the complete differential leukocyte count, per the complete-blood-count results-based reflex criteria of our laboratory. A blood smear processed only for the PLT scan produces only the PLT screen for review by the technologist.

Initially, we analyzed data from individual screens of the DM96 with regard to the total number of true positives and the total number of false negatives, to determine sensitivity. We considered a result from the DM96 to be a true positive if the analyzer detected and/or displayed 1 or more PLT clumps and/or 1 or more fibrin strands. In contrast, we considered a result from the DM96 to be false negative when it did not detect and display a positive finding of 1 or more PLT clump(s) or 1 or more fibrin strand(s) confirmed by microscopic review. Then, we repeated the same analysis after combining the data from 2 or more screens in an attempt to optimize the sensitivity of the DM96 for detecting PLT clumps. We calculated the sensitivity using the following formula.

\[
\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100
\]

We analyzed the data on relative distribution of PLT clumps on different parts of the blood smear with respect to the percentage of smears showing PLT clumps. We initially compared individual parts of the smears and then combined the observations from 2 or more parts, in an attempt to maximize the sensitivity.

Results

The results of the DM96 data analysis are shown in Table 1. The DM96 had a sensitivity of 40.4% for automatic detection of PLT clumps. We achieved a variable but higher level of sensitivity when the result-review process included visual examination of all 3 displays of the DM96 for PLT clumps. Visual examination of the entire WBC display and the entire PLT display yielded the highest sensitivity of 82.8%.

The second set of data on relative distribution of PLT clumps discovered in various parts of peripheral blood smears is shown in Table 2. The actual number and the fraction of specimens that revealed PLT clump(s), fibrin strand(s), or both, is shown in Table 2 for individual fields on the peripheral blood smear and for the combined data from 2 or more fields. The relative distribution of PLT clumps on the smears fulfilled the second goal of the study, which was to determine whether the traditional
practice of examining only the feather edge for PLT clumps is adequate for detecting the presence of PLT clumps in peripheral blood smears.

Qualitative PLT estimates from microscopic review of smears included “increased with clumps” in 9 (8.8%), normal with clumps” in 74 (72.5%), to “decreased with “clumps” for 11 of 102 smears (10.8%). We reported a qualitative PLT estimate of “normal or increased with clumps” for 8 of 102 smears (7.8%) because of the difficulty in differentiating “high normal” from “slightly increased” in the presence of clumps. We observed fibrin strands in addition to PLT clumps on 13 of 102 smears (12.7 %) via microscopic review and on 15 of 100 smears via the DM96 (15.0%).

Table 1. Analysis of Platelet Clumps Data Obtained From Various Screens of the CellaVision DM96

<table>
<thead>
<tr>
<th>PLT Clumps Data From Various Screens of the DM96</th>
<th>True Positive</th>
<th>False Negative</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT clumps detected/identified/recorded as PLT aggregates below the differential leukocyte count results on the WBC screen (DM96)</td>
<td>40</td>
<td>59</td>
<td>40.4%</td>
</tr>
<tr>
<td>DM96 + PLT clumps visualized by the technologist in the differential-leukocyte-count results area (DM96-W)</td>
<td>55</td>
<td>44</td>
<td>55.6%</td>
</tr>
<tr>
<td>DM96 + PLT clumps visualized by the technologist on the RBC morphology screen (DM96-R)</td>
<td>68</td>
<td>31</td>
<td>68.7%</td>
</tr>
<tr>
<td>DM96 + DM96-W + DM96-R</td>
<td>69</td>
<td>30</td>
<td>69.7%</td>
</tr>
<tr>
<td>PLT clumps visualized by the technologist on the PLT screen</td>
<td>73</td>
<td>26</td>
<td>73.7%</td>
</tr>
<tr>
<td>DM96 + PLT screen</td>
<td>80</td>
<td>19</td>
<td>80.8%</td>
</tr>
<tr>
<td>DM96 + DM96-R + PLT screen</td>
<td>81</td>
<td>18</td>
<td>81.8%</td>
</tr>
<tr>
<td>DM96 + DM96-W + PLT screen</td>
<td>82</td>
<td>17</td>
<td>82.8%</td>
</tr>
<tr>
<td>DM96 + DM96-W + DM96-R + PLT screen</td>
<td>82</td>
<td>17</td>
<td>82.8%</td>
</tr>
</tbody>
</table>

PLT, platelet; DM96, CellaVision DM96.

Table 2. Relative Distribution of PLT Clumps on Different Parts of the Blood Smears

<table>
<thead>
<tr>
<th>Part Examined for PLT Clumps and Fibrin Strands</th>
<th>Specimens Showing PLT Clumps, Fibrin Strands, or Both</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Readable area only</td>
<td>86 (84.3)</td>
<td></td>
</tr>
<tr>
<td>Feather edge only</td>
<td>87 (85.3)</td>
<td></td>
</tr>
<tr>
<td>Lateral edge 1 only</td>
<td>79 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Lateral edge 2 only</td>
<td>82 (80.4)</td>
<td></td>
</tr>
<tr>
<td>Lateral edge 1 + Lateral edge 2</td>
<td>84 (82.4)</td>
<td></td>
</tr>
<tr>
<td>Feather edge + Readable area</td>
<td>101 (99.0)</td>
<td></td>
</tr>
<tr>
<td>Feather edge + Readable area + Lateral edges</td>
<td>102 (100)</td>
<td></td>
</tr>
</tbody>
</table>

n = 102.

Discussion

We achieved the highest level of sensitivity of 82.8% when the reviewer looked for PLT clumps/fibrin strands on the entire WBC screen (ie, the area covered by the images of all cells on the WBC screen) as well as the entire PLT screen (all 9 sections). Additional review of the RBC screen for PLT clumps/fibrin strands did not increase the sensitivity. Automatic detection and display of PLT clumps/aggregates on the lower part of the WBC display via the DM96 yielded a sensitivity of only 40.4%. However, the sensitivity increased to 80.8% by additional review of the PLT screen for PLT clumps/fibrin strands and to 81.8% by additional review of the PLT and RBC screens for PLT clumps/fibrin strands. A review of only the PLT display for PLT clumps/fibrin strands yielded a sensitivity of 73.7%. Although we did not formally investigate it and do not show the resulting data herein, the sensitivity of detecting PLT clumps on peripheral blood smears using the DM96 turned out to be approximately 25.0% higher on smears prepared by the automated smear maker compared with the manually prepared smears. However, we can derive no valid conclusion about this difference between the 2 types of smears because manually prepared smears represented a very small fraction (only 12.1%) of the total number of smears that we evaluated.
To our knowledge, there are neither data on a comparative image analysis system in clinical use nor comparable published study results regarding the DM96 to which we can compare our findings. However, the flagging efficiencies for PLT clumps for different automated whole blood analyzers, as reported by Sandhaus et al.\(^9\) based on very limited data (a total of 6 specimens), range between 33% and 67%—this is much lower than the maximum attained in our study. The highest sensitivity, 82.8%, that we achieved in our study is less than the desirable level of 100%, most likely because the DM96 does not scan the entire smear for PLT clumps/fibrin strands and presents only selected fields of the smear for review. Neither the feather edge nor the lateral edges of the smear are routinely included in the DM96 display. The desired sensitivity level of nearly 100% may be achievable via an automated-image-analysis system such as the DM96 if it scans the entire smear at an appropriate magnification.

A review of the data on the relative distribution of PLT clumps on different parts of the peripheral blood smear in Table 2 reveals that we achieved the highest positive yield of 100% by examining all 3 fields (feather edge, readable area, and lateral edges) of the smear for PLT clumps. Also, we achieved a positive yield of 99% by examining just 2 areas, namely, the feather edge and the readable area, and we achieved a yield of 85.3% when we examined only the feather edge for PLT clumps. These findings indicate that for detecting PLT clumps on peripheral blood smears, examining only the feather edge is inadequate.

**Conclusion**

The highest sensitivity of 82.8% for the detection of PLT clumps/fibrin strands in blood smears via the DM96 is achievable by examining the entire WBC and the entire PLT displays. Microscopic examination of the peripheral blood smear for PLT clumps yields a sensitivity of 99% when the feather edge and the readable area of the smears are examined; this process yields a sensitivity of 100% when the technologist reviews the feather edge, readable area, and both lateral edges of smears. Laboratory technologists should examine, at minimum, the feather edge and the readable area of each smear.

**Acknowledgments**

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**References**


