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Original Article

Platelet count estimation using the CellaVision DM96 system

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Abstract

Introduction: Rapid and accurate determination of platelet count is an important factor in diagnostic medicine. Traditional microscopic methods are labor intensive with variable results and are highly dependent on the individual training. Recent developments in automated peripheral blood differentials using a computerized system have shown many advantages as a viable alternative. The purpose of this paper was to determine the reliability and accuracy of the CellaVision DM 96 system with regards to platelet counts. **Materials and Methods:** One hundred twenty seven peripheral blood smears were analyzed for platelet count by manual microscopy, an automated hematology analyzer (Beckman Counter LH 780 or Unicel DXH 800 analyzers) and with the CellaVision DM96 system. Results were compared using the correlations and Bland-Altman plots. **Results:** Platelet counts from the DM96 system showed an R^2 of 0.94 when compared to manual platelet estimates and an R^2 of 0.92 when compared to the automated hematology analyzer results. Bland-Altman plots did not show any systematic bias.

Key words: Digital pathology, method comparison, platelet estimation

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INTERPRETATION

The overall performance of the DM96 system for platelet counts was similar to both automated hematology analyzer and manual platelet estimates.

BACKGROUND

Rapid and accurate determination of platelet counts is an important factor in diagnostic pathology. Platelet counts are generally performed by automated analyzers using the coulter counter technology. These automated hematology analyzers usually provide accurate platelet counts with generally good precision; however, in some clinical situations interference with the automated count can occur, requiring a manual method of platelet estimation. Situations requiring manual platelet counts include the presence of micro clots, platelet aggregates, platelet satellitism, and red cell fragmentation.[1]

Recent developments in automated peripheral blood differentials using the computerized systems have allowed platelet estimation by scanning of digitized peripheral blood film images.[2] CellaVision DM96 is one such system utilized in the preliminary determination of differential counts on the peripheral blood or body fluid smears. The DM96 system has been shown previously to be valuable in determining leukocyte differentials[3-5] as well as analyzing RBC morphologies[6-8] in various clinical settings.

The aim of this study was to determine the reliability and accuracy of the CellaVision DM96 system with regards to platelet count determination by comparing CellaVision

DM96 platelet count estimates with manual microscopic estimates from the same slides as well as with platelet estimates by an automated analyzer.

MATERIALS AND METHODS

This study was part of the departmental method validation process and all samples included in this study were anonymized. This process is exempted from ethics review under institutional policies.

Blood Samples

Peripheral blood smears (PBS) of 127 patients from five medical centers (Foothills Medical Center, Alberta Children's Hospital, Rockyview General Hospital, Peter Lougheed Center, Diagnostic and Scientific Center) located in the greater Calgary (Alberta, Canada) area were used. The elapsed time between venipuncture and sample analysis was within 12 h.

Automated Analyzer Platelet Counts

Automated platelet counts (as part of complete blood counts) were performed utilizing automated hematology analyzers (LH 780 or Unicel DXH 800, Beckman Coulter, Brea, CA). These analyzers use electrical impedance to determine numbers of various cellular elements. Each cellular component (WBC, RBC or Platelet) generates a channelized pulse that is proportional to its size and volume, which are sorted based on the size to determine final counts for each cellular component. The pulse with a volume between 2-20 fL were considered and counted as platelets. Coulter analyzers will provide "flagging" if abnormal platelet size (i.e., giant platelets or platelet clumping) is encountered, which will then prompt the technologist to review a slide (standard microscopy or utilizing CellaVision DM96 software). Automated slide makers (LH 780 Beckman Coulter Brea, CA) were used to prepare PBS, which was stained with the Wright-Geimsa stain.

Manual Platelet Estimation

The PBS slides were independently examined by two experienced technologists (of a total of 10 technologists reading slides for this study) for platelet estimation and morphology (giant platelets, platelet clumps etc.) assessment. Platelet estimation was made according to established laboratory procedures. Briefly, the PBS was examined under a ×100 oil objective. An area where red cells were not overlapping was selected and the number of platelets counted under ×100 magnification in 10 fields (to account for uneven distribution of platelets in the peripheral blood smear) were averaged and then multiplied by 20,000 to get the estimated platelet count/uL. This method has been reported to be more accurate than an alternative method of manual platelet estimation.[9]

DM96 System

The CellaVision® DM96 system is designed to automatically perform preliminary differential counts on PBS or other body fluids. The analyzer pre-classifies the white blood cells, pre-characterizes parts of the red morphology and provides functionality for platelet estimation. With CellaVision® DM96 it is possible to capture digital images of custom defined areas of an interesting specimen, creating a digital slide. The digitized system gives a useful overview of the sample and allows for discussion between physicians regardless of physical location. Regions of interest can be tagged, comments added and exported into presentations, and educational material. The system analyzes and performs complete differentials at the rate of up to 35 slides/h.[10] For our analysis, only the platelet count data were used.

Platelet Evaluation Utilizing CellaVision DM96 System

CellaVision provides the technologist with an equivalent slide area corresponding to 8 microscopic high power fields (HPF). This overview image is divided into grid squares and the technologist's counts the number of platelets in all of the grid squares [Figure 1]. The average number of platelets/HPF value is obtained and multiplied by a pre-determined platelet estimate factor to determine the final platelet estimate count/10E^9/L. Platelet morphology was evaluated as described in manual method (above). A specific timing study, to determine the efficiency between manual and CellaVision DM96 platelet estimations was not performed *per se*; however, CellaVision DM96 was time efficient (23%), compared to standard microscopic examination of PBS in performing overall manual WBC differential counts and/or RBC morphology (data not shown).

Statistical Analysis

Analyses were performed using an Excel spreadsheet (Microsoft, Redmond, Washington). For each case, from the manual platelet estimation, the

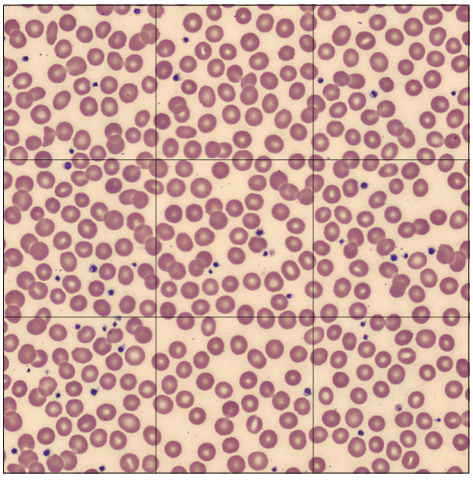

Figure 1: Example screenshot from the CellaVision DM96 system showing grid lines to aid in platelet estimation

automated analyzer platelet count and the CellaVision DM96 system platelet count estimates were entered into the spreadsheet. Correlation and Bland-Altman plots were used to compare CellaVision DM96 system results with the manual platelet estimates with the results of the hematology analyzer.

RESULTS

Comparison of the CellaVision DM96 system with manual platelet estimation and with an automated analyzer showed R^2 values of 0.94 [Figure 2] and 0.92 [Figure 3] respectively. The plot of the difference between the DM96 system and manual method values against their means according to the Bland and Altman design showed that the difference mean was − 3.67 with a standard deviation of 38.68, and with 92.9% of differences were within the agreement limits [mean ± 2SD; Figure 4]. The Bland and Altman plot for DM96 and the automated analyzer comparison showed a difference mean of 1.38 with a standard deviation of 46.40, and with 95.2% of the differences were within the agreement limits [mean ± 2SD; Figure 5]. Platelets morphology between standard microscopic examination and CellaVision DM96 evaluation did not show any significant disparity (data not shown).

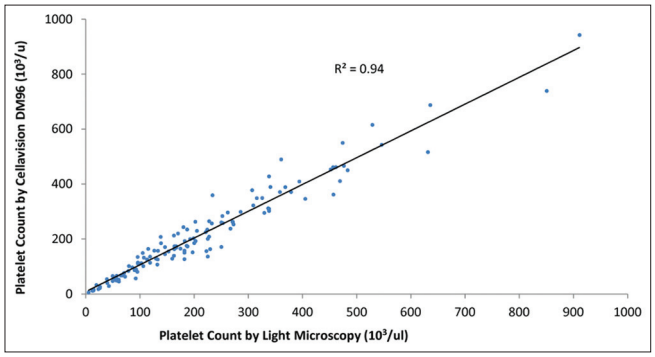

Figure 2: Correlation of manual platelet counts with CellaVision DM96 platelet counts

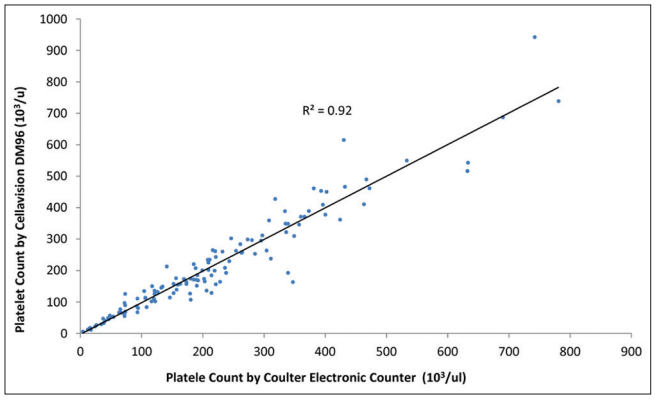

Figure 4: Correlation of automated hematology analyzer results with CellaVision DM96 platelet counts

CONCLUSIONS

Platelet count estimates obtained via the CellaVision DM96 system compared very well with both manual estimates and hematology analyzer results on the same cases. Although, automated analyzer methods will undoubtedly remain the principle diagnostic modality for platelet count estimates, our study suggests that when PBS are analyzed with the CellaVision DM96 system, the automated platelet count estimates are reliably accurate. In routine clinical practice, two relevant and important components of platelet morphology are considered to be giant platelets and platelet clumps. Miscroscopic examination of slides by a trained technologist is mandated, if automated analyzer flags the possible presence of these features in a given specimen. Our study did not find any discrepancy between standard microscopic examination and CellaVision DM96 evaluation for these morphological features.

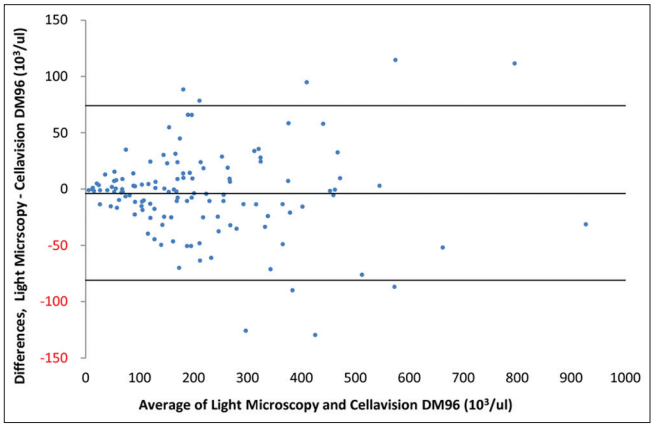

Figure 3: Difference versus mean plots for CellaVision DM96 and manual platelet counts according to the Bland and Altman design. The middle solid line is the mean of the difference; the outer solid lines are the upper and lower limits of agreements (mean ± 2SD)

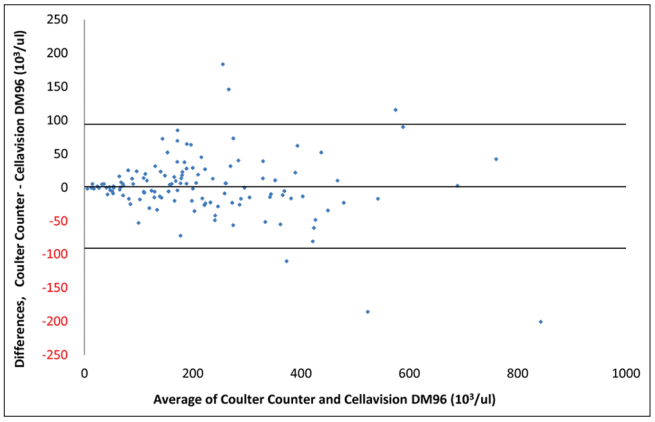

Figure 5: Difference versus mean plots for CellaVision DM96 and automated hematology analyzer platelet counts according to the Bland and Altman design. The middle solid line is the mean of the difference; the outer solid lines are the upper and lower limits of agreements (mean ± 2SD)

Although manual platelet estimates are necessary in some circumstances, the coefficient of variation, in our experience, can vary from 50% in platelet counts of 50-15% with normal platelet counts. Some of this variation can likely be attributed to observer variability in the selection of fields for counting platelets in addition to technical variation in blood smear preparation. The CellaVision DM96 system offers several potential advantages in this regard. First, because it is fully automated, it eliminates the need for technologists to perform time-consuming manual platelet estimates. Second, consistency in scanning the defined area of the slide and provision of a grid could be expected to enhance the reproducibility of estimates over successive samples in clinical practice.

In our own institution, we have additionally found that the use of this digitized system has also been beneficial in training laboratory technologists and improving their competency and proficiency in performing platelet estimates.

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