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Original Article

Performance of the CellaVision® DM96 system for detecting red blood cell morphologic abnormalities

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Abstract

Background: Red blood cell (RBC) analysis is a key feature in the evaluation of hematological disorders. The gold standard light microscopy technique has high sensitivity, but is a relativity time-consuming and labor intensive procedure. This study tested the sensitivity and specificity of gold standard light microscopy manual differential to the CellaVision® DM96 (CCS; CellaVision, Lund, Sweden) automated image analysis system, which takes digital images of samples at high magnification and compares these images with an artificial neural network based on a database of cells and preclassified according to RBC morphology. **Methods:** In this study, 212 abnormal peripheral blood smears within the Calgary Laboratory Services network of hospital laboratories were selected and assessed for 15 different RBC morphologic abnormalities by manual microscopy. The same samples were reassessed as a manual addition from the instrument screen using the CellaVision® DM96 system with 8 microscope high power fields (×100 objective and a 22 mm ocular). The results of the investigation were then used to calculate the sensitivity and specificity of the CellaVision® DM96 system in reference to light microscopy. **Results:** The sensitivity ranged from a low of 33% (RBC agglutination) to a high of 100% (sickle cells, stomatocytes). The remainder of the RBC abnormalities tested somewhere between these two extremes. The specificity ranged from 84% (schistocytes) to 99.5% (sickle cells, stomatocytes). **Conclusions:** Our results showed generally high specificities but variable sensitivities for RBC morphologic abnormalities.

Key words: Image analysis, method validation, peripheral blood smears

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INTRODUCTION

Microscopic examination of peripheral blood films is time-consuming, labor intensive, requires highly trained staff and remains subject to significant statistical variance.[1] Recent advances in laboratory medicine have allowed for automated devices, like the CellaVision® DM96 (CCS; CellaVision, Lund, Sweden), to screen for specific cell abnormalities at a much more efficient rate than without automation.[2-7] Previous evaluations of these types of systems have shown good correlations with the manual differentiation of normal cells.[4,8,9]

Automated red blood cell (RBC) analysis and screening therefore presents clinical laboratories with the potential to process large sample volumes, while ensuring a high accuracy rate in diagnosis. In particular, routine

identification of the absence or presence of abnormal morphological features in RBC can be an important diagnostic tool for many hematologic diseases. For example, the presence of acanthocytes in blood smears may indicate hemolytic anemia of severe liver disease.[10] However, microscopic analysis is still needed when abnormal cells are present[11] because automated analyzers offer limited morphological information for RBC's.[12] Some institutions may choose to "autoverify" the results from the CellaVision® DM96 into their Laboratory Information System. However, at Calgary Laboratory Services (CLS) the RBC morphologic abnormalities identified by the system are sent for medical laboratory technologist (MLT) or pathologist review. Therefore at CLS, the CellaVision® DM96 is primarily used as a screening tool for RBC morphologic abnormalities.

In this paper, we assessed the ability of the CellaVision® DM96 in identifying RBC morphologic abnormalities.

METHODS

CellaVision® DM96

The CellaVision® DM96 is an automated system for *in vitro* diagnostic practice. The system scans a user-defined portion of a microscopy slide and automatically locates and presents images of cells on blood smears. A peripheral blood application is specified for differential count of white blood cells, classification of RBC morphology and platelet estimation. The CellaVision® DM96 system overlays the digitized image with a grid to facilitate accurate cell counting. The operator recognizes and verifies the recommended classification of all cells according to types specified in a preexisting database.[13] For RBC morphology, the CellaVision® DM96 system scans one zone equivalent to eight fields using a ×100 objective. The CellaVision® DM96 system is outfitted with four flag levels to discriminate between six morphological abnormalities of size and color of RBCs including hypochromasia, polychromasia, microcytosis, macrocytosis, anisocytosis and poikilocytosis,[3] and has the ability to characterize several more, many of which are included in this study.[13] Only the RBC abnormalities that are routinely screened at CLS were examined.

Selection Criteria

Peripheral blood smears performed as part of routine medical care were collected from five hematology labs in Calgary, Alberta, Canada. Four of the centers are acute care facilities: Foothills Medical Center (50 smears), Peter Lougheed Centre (50 smears), the Rockyview General Hospital (35 smears) and the Alberta Children's Hospital (37 smears). The fifth lab is the Diagnostic and Scientific Center (40 smears), which collects and analyses samples from the Calgary community and surrounding towns in the Southern Alberta Region. The total number of patients for our study is 212. However, some of the specific RBC abnormalities were not identified in CellaVision® DM96 run samples, such as malaria parasites, Basophilic Stippling, Burr Cells and Howell–Jolly Bodies in the count for that sample. The samples testing for malaria are not further analyzed in the Hematology department and are then forwarded to microbiology for further testing. Therefore, these individual results were excluded, reducing the number of samples to 198 for some RBC abnormality counts.

The selection criteria for the study identified "abnormal" RBC morphological samples at each site. These samples were identified by randomly selecting "abnormally" screened RBC morphology samples by the initial screening device, the Coulter LH 780 analyzer. The analyzer identifies nonspecific RBC abnormalities such as low/high hemoglobin or mean corpuscular volume. These nonspecific abnormalities trigger the analyzer to automatically create a peripheral smear on a glass slide. These slides were then chosen manually for specific RBC abnormalities. Abnormal samples identified with RBC morphological defects were collected until a preselected amount of samples was met. Once this amount was reached the slides were screened by the CellaVision® DM96 and also by the MLTs at each site.

Smears and Stains

The blood samples from each site were collected in 5 ml ethylenediaminetetraacetic acid tubes. Slide smears of the abnormal slides were made using an automatic Coulter LH slide maker and then stained with Wright-Giemsa by the Coulter LH slide stainer. Each slide was selected for only one specific abnormality. As a result, each slide acted in effect as a negative control for every other abnormality.

Differential Counts

Each site performed manual differentiation using standard microscopic techniques on each of the slides. For standard RBC morphology identification, the smears were evaluated, as per CLS hematology department standards, by using a ×100 objective lens scanning 10 separate fields [Table 1].[14] These standards were based on hematology reference materials[15] and then established using International Society for Laboratory Hematology guidelines for suggested action following automated complete blood count differential analysis.[16] Each slide was scored at each individual site by two experienced MLTs. In cases of observer disagreement, a third MLT served as a tie-breaker.

All of the sites had experienced MLTs (25+ year's experience) evaluate the slides. The results were reported as either present or absent for 15 different RBC morphological abnormalities: Acanthocytes, basophilic stippling, burr cells, Howell–Jolly bodies, ovalocytes, Pappenheimer bodies, polychromasia, RBC agglutination,

rouleaux, schistocytes, sickle cells, spherocytes, stomatocytes, target cells and tear drop cells.

The same slides were analyzed by the CellaVision® DM96 system. Each slide was scored and data was collected using the same criteria as the manual differential method where RBC morphology abnormalities were reported as present or absent. The classification of any of the RBC abnormalities studied is a manual addition from the instrument screen and is not automatic. The CellaVision® DM96 and glass slide classifications were then compared in truth tables and sensitivities and specificities calculated. A representative digitized field from the CellaVision® DM96 system is shown in Figure 1.

RESULTS

Results from the classification into normal and abnormal were sorted into the categories in Table 2. In regards to sensitivity, we observed a wide range of results in identifying RBC morphological abnormalities. For some RBC abnormalities, such as stomatocytes and sickle cells, there was a 100% sensitivity rate. However, for RBC agglutination and burr cells, there were low sensitivity rates of 33% and 47%, respectively. The other abnormalities ranged in sensitivity from 56% to 84%, respectively.

In regards to specificity, we found more consistent results among the RBC abnormalities. The range of specificity was from 84% for schistocytes to 99.5% for both sickle Cells and stomatocytes with the other RBC abnormalities within these two values. The majority of the RBC abnormalities were in the upper 90%.

DISCUSSION

We observed generally very good specificities but variable sensitivities when the CellaVision® DM96 system was used to classify RBC morphologic abnormalities when compared to traditional glass slide microscopy. There is only very limited existing literature addressing this question, and reported results have varied widely. One prior study compared a different analytical system, the Diffmaster Octavia, with the CellaVision® DM96. The results showed impressive sensitivity and specificity results.[6] However, the two different systems were compared only to each other and not to a proper reference method such as the industry gold standard manual differential. Another study showed that when

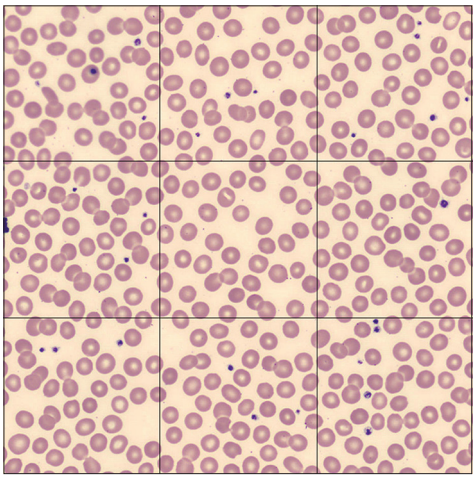

Figure 1: Example screenshot from the CellaVision® DM96 system showing normal red blood cells

Table 1: RBC morphologic abnormalities examined in this study

Abnormality	Definition of present (%[a])
Acanthocytes	>5
Basophilic stippling	>2
Burr cells	>10
Howell-Jolly bodies	Present in any amount[b]
Ovalocytes	>10
Pappenheimer bodies	Present in any amount
Polychromasia	>5
RBC agglutination	Present in any amount
Rouleaux	>40
Schistocytes	>2
Sickle cells	Present in any amount
Spherocytes	>2
Stomatocytes	>10
Target cells	>10
Tear drop cells	>2

[a]Percentage of cells seen per ×100 oil immersion field except rouleaux, [b]Present indicates a morphological feature that is moderate or markedly increased. RBC: Red blood cell

Table 2: Sensitivities and specificities of 15 RBC morphologic abnormalities assessed through the CellaVision® DM96 system as compared to glass slide assessment

Abnormality	Sensitivity %	Specificity %
Acanthocytes	75.0	97.0
Basophilic stippling	62.0	99.0
Burr cells	47.0	96.0
Howell-Jolly bodies	84.0	94.0
Ovalocytes	81.0	89.0
Pappenheimer bodies	82.0	96.0
Polychromasia	73.0	87.0
RBC agglutination	33.0	99.0
Rouleaux	56.0	97.0
Schistocytes	60.0	84.0
Sickle cells	100.0	99.5
Spherocytes	57.0	91.0
Stomatocytes	100.0	99.5
Target cells	83.0	97.0
Tear drop cells	81.0	95.0

RBC: Red blood cell

direct microscopy was used as the reference method against, which the results of the CellaVision® DM96 were compared, with all discrepant results included in the analysis, the specificity was 82–93% and sensitivity was 25–91%, – which was similar to the results of this study.[4] Finally, another study reported that blood cell and platelet morphology was judged to be adequate; however it was not specific for RBC morphological abnormalities.[11]

There are several possible limitations in this study. First, the MLTs participating at each laboratory involved in the study only analyzed the slides from their individual sites. As a result, although the slides were presented in a blinded fashion to the MLTs, there is a large probability that the same technologist manually read the slide and reclassified the CellaVision® DM96 results. This may have enhanced the correlation between the automated and manual classification methods.

In addition, the selection of peripheral blood smears for this study is not random as slides only with abnormalities were selected. The percentage of abnormal smears is low in routine hematopathology practice and a random sample would evaluate very low numbers of abnormal smears. Additionally, the testing of normal blood samples has already been performed and the results have already shown the CellaVision® DM96 to be reliable and accurate.[6]

One interesting observation, corroborated by our study, was that RBC agglutination sensitivity has tested poorly in other CellaVision® DM96 validation studies. Billard *et al*., has previously shown decreased sensitivity in platelet aggregates/"thrombocytic agglutinates" and RBC agglutination.[12] This study, and the Billard *et al*. study, may indicate a possible limitation in sensitivity for RBC agglutination in the CellaVision® DM96.

CONCLUSIONS

This study shows that the CellaVision® DM96 has acceptable specificity, but lacks consistency for sensitivity. A future test featuring sensitivity of RBC agglutination, and other low scoring RBC morphologic abnormalities, combined with a larger number of samples may be warranted to confirm these results.

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