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Brief Report

Accuracy of the CellaVision DM96 platform for reticulocyte counting

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Abstract

Context: Many hematology laboratories have adopted semi-automated digital platforms for routine use and the evidence supporting their use is increasing. **Aims:** The CellaVision platforms are among the most thoroughly studied digital hematology platforms; we wished to determine the accuracy of CellaVision for reticulocyte counting. **Design, Materials and Methods:** We compared reticulocyte counts performed manually, using the Beckman Coulter LH750 automated analyzer and with the CellaVision DM96 platform. We analyzed the results for pair-wise correlation and bias, and precision. **Statistical Analyses Used:** Analyses were performed using Statistical Package for the Social Sciences software (SPSS), including Spearman's rho correlation coefficient, Friedman's two-way Analysis Of Variance (ANOVA) for comparison of distributions; bias was compared by way of mean and standard deviation. **Results:** The CellaVision reticulocyte counts correlated most strongly with those of the analyzer (often considered the benchmark test); the reticulocyte count distributions were noted not to be significantly different from each other across all three methods. The mean and standard deviation of bias were lowest in the comparison of CellaVision and LH750 counts. **Conclusions:** Our data provide additional support for the accuracy of digital hematology applications using the CellaVision DM96 platform.

Key words: Accuracy, CellaVision, digital hematology, laboratory automation, reticulocyte

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INTRODUCTION

Whole-slide imaging and digital image analysis have become commonplace in laboratory medicine and for the past decade many high-volume hematology laboratories have adopted semi-automated digital platforms for routine use.[1] The CellaVision platforms are among the most thoroughly studied digital hematology platforms in use. Indeed, a review of the literature highlights the rigorous evaluation that the CellaVision system has undergone by multiple groups and from multiple perspectives: Several studies have supported the accuracy and precision of CellaVision for the purposes of the leukocyte differential;[2-15] others have highlighted the accuracy and precision of CellaVision in the analysis of red cell parameters;[3-5,11,13,16] others still have validated CellaVision for use in platelet counting and morphological assessment.[3,11,13,17] In reviewing the literature, only a

single publication could be identified pertaining to the evaluation of digitized slide analysis for the purpose of reticulocyte counting;[18] however, no CellaVision-specific data was identified.

Reticulocytes are immature erythrocytes released into the circulation just prior to the completion of their maturation; they can be identified by the presence of two or more basophilic intracytoplasmic granules corresponding to residual erythrocyte ribosomes[19] (and usually only visible by supravital staining, with new methylene blue for example). The clinical utility of the identification and quantification of reticulocytes in the peripheral blood is considerable; reticulocytes are useful in the diagnostic work-up of anemia, in the subclassification of macrocytosis in certain contexts and in the assessment of response to treatments for anemia.[19-22] While the earliest identification techniques relied upon manual counts,[19] in recent decades automated platforms have become the mainstay of reticulocyte assessment given more recent evidence of a lack of precision in manual assessments.[19,23] Nevertheless, a manual reticulocyte count remains a useful yardstick in the context of quality assurance.

As part of our laboratory's recent adoption of a holistic digital hematology system, we rigorously tested the CellaVision platform to ensure that it might be used under the most stringent clinical validation but also to its full methodological potential. Part of this validation process included a comparison of reticulocyte counts from manual, automated analyzer and CellaVision testing.

MATERIALS AND METHODS

This study was considered to constitute a quality assurance and laboratory validation project by our institution; and therefore, did not require formal research ethics board approval, as outlined by the Alberta Research Ethics Community Consensus Initiative (ARECCI) ethics guidelines.[24] From each of the four hospital-based hematology laboratories in our laboratory network, series of consecutive patient blood samples were assessed; the number of sample selected was in keeping with previous data concerning correlation and sample size.[25]

Standard analyzer testing was performed on a Beckman Coulter LH750 automated hematology analyzer (Beckman Coulter Canada LP) using the integrated new methylene blue approach, as previously described.[26] The CellaVision (DM96, CellaVision, Lund, Sweden) and Manual percent reticulocyte counts were performed using supravital stained slides (produced by mixing equal aliquots of ethylenediaminetetraacetic acid-collected whole blood with new methylene blue) of the same samples analyzed by the LH750 platform. Manual percent reticulocyte counts were performed by a group of technologists from the individual sites of origin and based on a 200-erythrocyte total count. The CellaVision counts were obtained through automated (pre-classification) analysis only. In this process, the new methylene blue stained peripheral smear slides used in the manual counts were scanned and interpreted by CellaVision as part of its usual peripheral blood smear interpretation algorithm (no reticulocyte-specific protocol currently exists as part of the fully automated CellaVision algorithms). After pre-classification, technologist-assisted reticulocyte enumeration was performed; this count was taken as the CellaVision percent reticulocyte count. In addition, we did not adjust the reticulocyte counts for the degree of anemia as, for the purposes of method validation, we did not feel that this would provide significant information.

Percent reticulocyte counts were compared from among the three methods and data analyzed (by way of Spearman's rho correlation coefficients, Friedman's two-way Analysis Of Variance (ANOVA), bias mean and standard deviation) using Statistical Package for the Social Sciences software (SPSS Version 20, IBM); *P* values of less than 0.05 were considered significant.

RESULTS

Twenty-five consecutive peripheral blood samples from each of our four hospital labs were included in the study sample (for a total of 100). Reticulocyte counts were recorded and plotted for all three methods [Figures 1, 2 and 3]. There was significant correlation between the results of each method by Spearman's rho coefficient testing [$P < 0.001$; Table 1]. Non-parametric testing for unrelated sample distributions was not significant by Friedman's two-way ANOVA ($P = 0.086$). R-squared values were calculated pairwise: LH750 vs. CellaVision $R^2 = 0.86$; manual count *vs*. LH750 $R^2 = 0.75$; manual count *vs*. CellaVision $R^2 = 0.78$. Pair-wise bias was also calculated and compared [Table 2] using absolute values (given that a "true" value was not assumed *a priori*); these pair-wise distributions were not significantly different by Friedman's two-way ANOVA ($P = 0.71$). Notably, the mean and standard deviation of bias in comparing the LH750 vs. CellaVision were lower than the respective values when manual counts were compared with both the LH750 and CellaVision counts.

DISCUSSION

The CellaVision digital hematology platform has a well-validated track record for use in routine hematological laboratory practice. Published studies have reported a median leukocyte differential count accuracy of 90.3% in comparison to reference manual differentials.[4-7,14] Others have reported a range of accuracy of pre-classification of erythrocyte morphological abnormalities from 65% to 98%, depending on the nature of the abnormality.[16] Still others

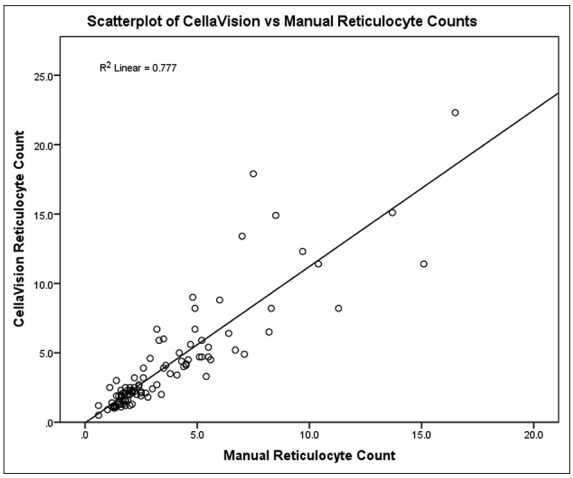

Figure 1: Scatterplot of CellaVision vs. manual % reticulocyte counts

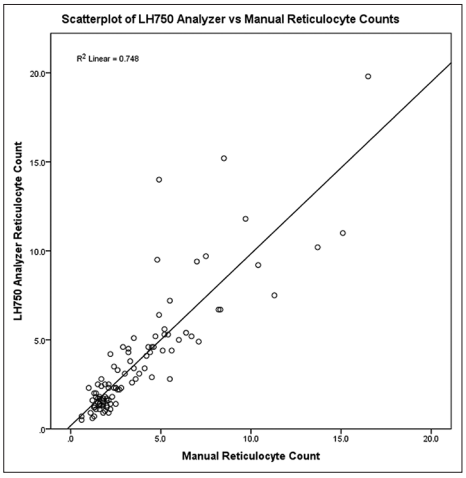

Figure 2: Scatterplot of LH750 analyzer vs. manual % reticulocyte counts

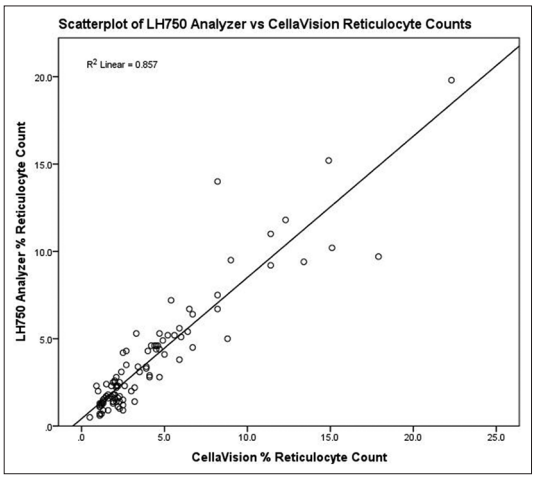

Figure 3: Scatterplot of LH750 vs. CellaVision % reticulocyte counts

have evaluated platelet-counting concordance relative to standards, noting a range of correlation coefficients from 0.92 to 0.94.[17] These data, in combination with evidence of potential substantial improvements in per-slide turn-around time,[5] make the CellaVision platform an attractive option for the purposes of semi-automation of peripheral smear morphology interpretation.

Table 1: Spearman's rho correlation coefficients for pair-wise comparisons of manual, LH750 analyzer and CellaVision % reticulocyte counts

Spearman's rho correlation coefficients			
	Manual reticulocyte count	**LH750 analyzer reticulocyte count**	**CellaVision reticulocyte count**
Manual reticulocyte count			
Correlation coefficient	-	0.873**	0.898**
P value (2-tailed)	-	0.000	0.000
LH750 analyzer reticulocyte count			
Correlation coefficient	0.873**	-	0.873**
P value (2-tailed)	0.000	-	0.000
CellaVision reticulocyte count			
Correlation coefficient	0.898**	0.873**	-
P value (2-tailed)	0.000	0.000	-

** Significant

Table 2: Pairwise bias

	Mean	**Std. deviation**
Manual count vs CellaVision count bias	1.047	1.5990
Manual count vs lh750 count bias	1.007	1.3641
LH750 count vs CellaVision count bias	0.870	1.2407

For high-throughput hematology laboratories, reticulocyte counts can be accurately and cheaply assessed using automated hematology analyzers, with excellent turnaround times.[26] In most cases, the use of CellaVision for the purposes of reticulocyte counting would likely not add a significant cost-benefit or turnaround time advantage (indeed, in our institution's experience, the average turnaround time for a CellaVision reticulocyte count is on par with that of a manual count; data not shown).

On the other hand, one can easily imagine scenarios in which reticulocyte counting using CellaVision might be a viable option. Let us consider, for example, the case of a small/rural low test-volume satellite laboratory without an available analyzer. When requested to perform a complete blood count (CBC) and reticulocyte count, rather than shipping the specimen, a peripheral smear could be produced and digitally scanned at the satellite site, the digital images (including one of a slide stained with new

methylene blue) could be forwarded electronically to a larger referral laboratory, at which point all peripheral blood parameters (including the standard CBC and reticulocyte counts), as well as morphologic assessment, could be assessed and the data subsequently relayed back to the referring satellite laboratory. In such a scenario, the CellaVision holistic approach to digitized hematology might be an attractive option. When one considers the relative cost of purchase of a small analyzer capable of assessing reticulocyte counts and a CellaVision scanner, the above solution might indeed be a viable option for some; such automated analyzers have capital costs on par or greater than a comparable CellaVision system (based on CAP TODAY Hematology Analyzer Platform Product Comparisons.[27]) Other potential benefits of the CellaVision system include real-time morphologically verifiable quality-assurance capabilities,[1,28] as well as the capacity for centralization of morphological expertise.[1]

REFERENCES

1. Pantanowitz L, Wiley CA, Demetris A, Lesniak A, Ahmed I, Cable W, *et al.* Experience with multimodality telepathology at the University of Pittsburgh Medical Center. J Pathol Inform 2012;3:45.
2. Amundsen EK, Urdal P, Hagve TA, Holthe MR, Henriksson CE. Absolute neutrophil counts from automated hematology instruments are accurate and precise even at very low levels. Am J Clin Pathol 2012;137:862-9.
3. Billard M, Lainey E, Armoogum P, Alberti C, Fenneteau O, Da Costa L. Evaluation of the CellaVision DM automated microscope in pediatrics. Int J Lab Hematol 2010;32:530-8.
4. Briggs C, Longair I, Slavik M, Thwaite K, Mills R, Thavaraja V, *et al.* Can automated blood film analysis replace the manual differential? An evaluation of the CellaVision DM96 automated image analysis system. Int J Lab Hematol 2009;31:48-60.
5. Ceelie H, Dinkelaar RB, van Gelder W. Examination of peripheral blood films using automated microscopy; evaluation of Diffmaster Octavia and Cellavision DM96. J Clin Pathol 2007;60:72-9.
6. Cornet E, Perol JP, Troussard X. Performance evaluation and relevance of the CellaVision DM96 system in routine analysis and in patients with malignant hematological diseases. Int J Lab Hematol 2008;30:536-42.
7. Kratz A, Bengtsson HI, Casey JE, Keefe JM, Beatrice GH, Grzybek DY, *et al.* Performance evaluation of the CellaVision DM96 system: WBC differentials by automated digital image analysis supported by an artificial neural network. Am J Clin Pathol 2005;124:770-81.
8. Lee LH, Mansoor A, Wood B, Nelson H, Higa D, Naugler C. Performance of CellaVision DM96 in leukocyte classification. J Pathol Inform 2013;4:14.
9. Linssen J, Jennissen V, Hildmann J, Reisinger E, Schindler J, Malchau G, *et al.* Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/secreting cells using the automated routine hematology analyzer XE-2100. Cytometry B Clin Cytom 2007;72:157-66.
10. Park SH, Park CJ, Choi MO, Kim MJ, Cho YU, Jang S, *et al.* Automated digital cell morphology identification system (CellaVision DM96) is very useful for leukocyte differentials in specimens with qualitative or quantitative abnormalities. Int J Lab Hematol 2013;35:517-27.
11. Rollins-Raval MA, Raval JS, Contis L. Experience with CellaVision DM96 for peripheral blood differentials in a large multi-center academic hospital system. J Pathol Inform 2012;3:29.
12. Smits SM, Leyte A. Clinical performance evaluation of the CellaVision Image Capture System in the white blood cell differential on peripheral blood smears. J Clin Pathol 2014;67:168-72.
13. Surcouf C, Delaune D, Samson T, Foissaud V. Automated cell recognition in hematology: CellaVision DM96 TM system. Ann Biol Clin (Paris) 2009;67:419-24.
14. Yamamoto T, Tabe Y, Ishii K, Itoh S, Maeno I, Matsumoto K, *et al.* Performance evaluation of the CellaVision DM96 system in WBC differentials. Rinsho Byori 2010;58:884-90.
15. Yu H, Ok CY, Hesse A, Nordell P, Connor D, Sjostedt E, *et al.* Evaluation of an automated digital imaging system, Nextslide Digital Review Network, for examination of peripheral blood smears. Arch Pathol Lab Med 2012;136:660-7.
16. Maenou I, Tabe Y, Bengtsson HI, Ishii K, Miyake K, Horiuchi Y, *et al.* Performance evaluation of the automated morphological analysis of erythrocytes by CellaVision DM96. Clin Lab 2013;59:1413-7.
17. Gao Y, Mansoor A, Wood B, Nelson H, Higa D, Naugler C. Platelet count estimation using the CellaVision DM96 system. J Pathol Inform 2013;4:16.
18. Piatnitskii AM, Medovyi VS, Parpara AA. Analysis of reticulocytes: Manual microscopy, flow analyzers or image analyzers? (analytical review)]. Klin Lab Diagn 2007:10-4.
19. Koepke JF, Koepke JA. Reticulocytes. Clin Lab Haematol 1986;8:169-79.
20. Brugnara C. Use of reticulocyte cellular indices in the diagnosis and treatment of hematological disorders. Int J Clin Lab Res 1998;28:1-11.
21. Brugnara C. Iron deficiency and erythropoiesis: New diagnostic approaches. Clin Chem 2003;49:1573-8.
22. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. JAMA 1999;281:2225-30.
23. Savage RA, Skoog DP, Rabinovitch A. Analytic inaccuracy and imprecision in reticulocyte counting: A preliminary report from the College of American Pathologists Reticulocyte Project. Blood Cells 1985;11:97-112.
24. Network ARECCIA. ARECCI Ethics Screening Tool. 2005.
25. Bates BT, Zhang S, Dufek JS, Chen FC. The effects of sample size and variability on the correlation coefficient. Med Sci Sports Exerc 1996;28:386-91.
26. Riley RS. Reticulocyte enumeration: Past and present. Lab Med 2001;32:599-608.
27. Pathologists CoA. CAP Today Hematology Analyzers Product Guides, 2013. CAP TODAY. 2013;2013
28. Horiuchi Y, Tabe Y, Idei M, Bengtsson HI, Ishii K, Horii T, *et al.* The use of CellaVision competency software for external quality assessment and continuing professional development. J Clin Pathol 2011;64:610-7.