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Published in:
Diagnostic Microbiology and Infectious Disease

DOI:
10.1016/j.diagmicrobio.2017.12.002

Publication date:
2018

License:
Unspecified

Document Version:
Final published version

Diagnostic Microbiology and Infectious Disease 90 (2018) 253–256

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Parasitology

Evaluation of the CellaVision DM96 advanced RBC application for screening and follow-up of malaria infection

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ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 15 November 2017
Accepted 2 December 2017
Available online 8 December 2017

Keywords:
CellaVision
Malaria
Diagnosis
Parasitemia

ABSTRACT

CellaVision DM96 is a digital cell morphology system for automated classification of white and red blood cells. CellaVision Advanced RBC application (ARBCA) pre-classifies RBC in 21 categories, including parasitized RBC, and allows re-classification by the operator. In this study, the performance of the software for detection of malaria and calculation of parasitemia was evaluated and compared to microscopy ($n = 40$). For CellaVision, both pre- and post-reclassification results were evaluated. Sensitivity was moderate, even post-reclassification (72%), due to low numbers of analyzed RBC and limited resolution of photographs. CellaVision results correlated with microscopy according to Passing-Bablok analysis, with slightly lower values for CellaVision. Within-run, between-run and inter-observer variability were acceptable. The low sensitivity of CellaVision ARBCA precludes its use as a screening technique for malaria. However, due to its good correlation with microscopy and short turn-around-times, it may be useful in follow-up of parasitemia. Larger studies are required to confirm these findings.

1. Introduction

Malaria is a global health problem, affecting in 2015 an estimated 212 million cases worldwide with 429,000 deaths (World Health Organization, 2016). Even though 90% of transmissions occur in the African continent, also Western countries where malaria is not present endemically cope with malaria infections due to immigration and international travel. Laboratory diagnosis of *Plasmodium* parasites in peripheral blood thick smears by optical microscopy is since long the diagnostic gold standard, with sensitivities ranging between 85% - 93% (Maguire et al., 2006; World Health Organization, 1988). Thick smears commonly yield higher sensitivities compared to thin smears, but positive results should be followed by analysis of peripheral blood thin smears for identification of *Plasmodium* species. Malaria rapid diagnostic tests (RDT) and PCR-based tests have an added value in malaria diagnosis. RDT have short turn-around-times, are easy to use and interpret and may be able to distinguish between *P. falciparum* and non-falciparum species, but sensitivity is limited. Furthermore, the detected malaria antigen is only slowly eliminated from the bloodstream and antigen may persist long after patient was treated and parasites were eliminated, making RDT unusable for follow-up of malaria infection. PCR-based tests have a higher sensitivity but may not be readily available and commercial tests are not always able to distinguish between different *Plasmodium* species. In addition, neither RDT nor PCR allow parasite density (parasitemia) counting. Therefore, microscopic review remains necessary. However, manual counting is time-consuming and prone to high inter-observer variabilities, which may be partially due to differences in counting method (Billo et al., 2013; Bowers et al., 2009).

The CellaVision DM96 (Lund, Sweden) is an automated digital cell morphology system and uses artificial neural network technology to locate, identify and pre-classify white blood cells and categorize red blood cells (RBC). The CellaVision Advanced RBC application (ARBCA) is a software tool that scans between 1500 and 3000 RBC's on every peripheral blood smear, photographs every individual RBC and supports RBC differential into 21 different morphological categories depending on size, shape, color and inclusions. The software recognizes red blood cells infected with parasites, including malaria, as one of the 21 categories and is able to calculate the percentage of infected RBC. The ARBCA categorizes every single RBC (pre-reclassification) and the operator subsequently manually adjusts the suggested category of RBC (=post-reclassification) if necessary. Results of RBC categories are reported semi-quantitatively in four flag levels (0, 1, 2 and 3), based on cut-offs established by the laboratory, as well as quantitatively as % of RBC (Fig. 1). In this way, CellaVision ARBCA may be able to facilitate and standardize the review of RBC for detection of malaria parasites and

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https://doi.org/10.1016/j.diagmicrobio.2017.12.002
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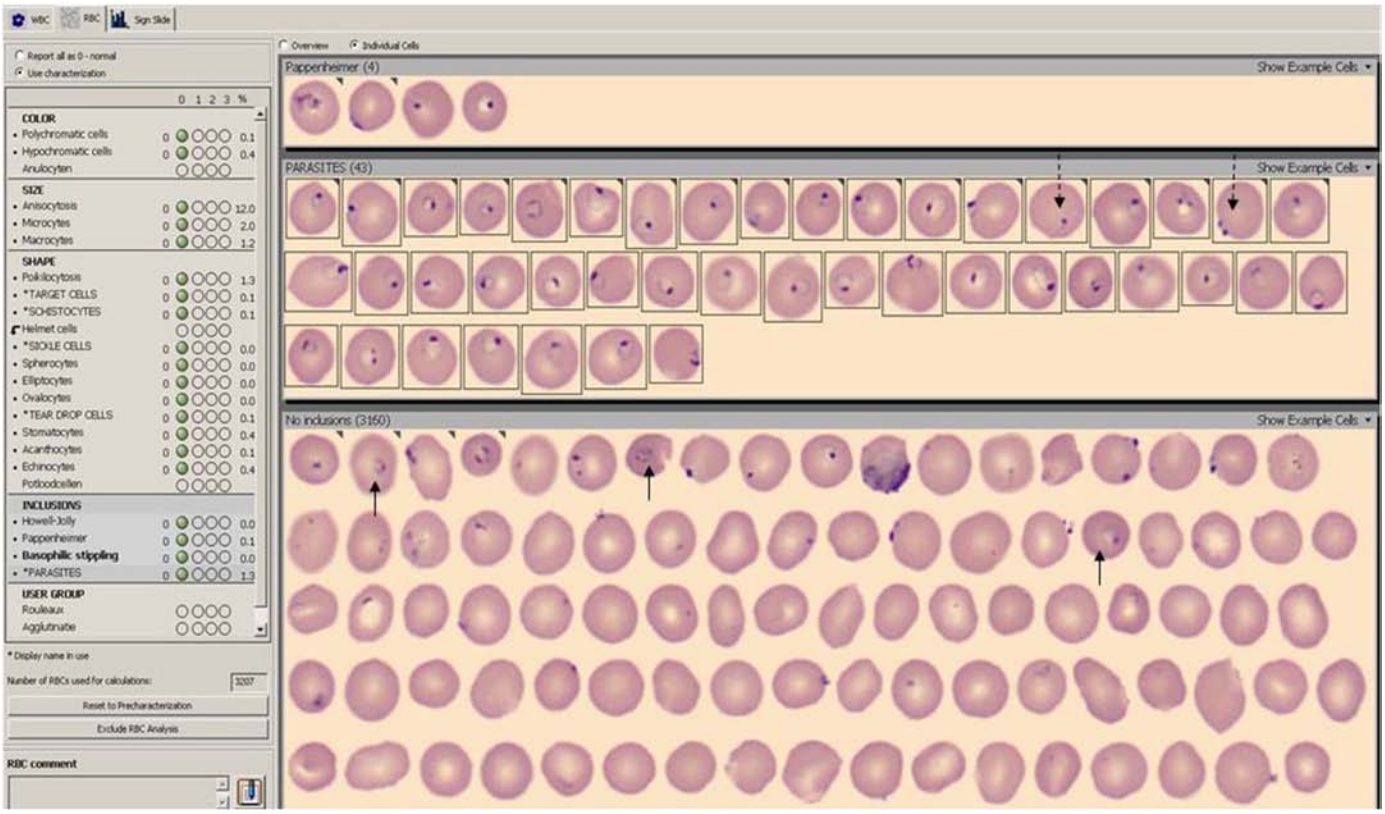

Fig. 1. Overview of the CellaVision Advanced RBC Application, which performs automatic acquisition and pre-classification of RBC in the category "parasites" (pre-reclassification). The operator is subsequently able to modify the suggested classification of every RBC (post-reclassification). Some false positive and false negative infected RBC, as classified by CellaVision, are indicated by a dotted and solid arrow respectively.

this application may be of use for diagnosis and follow-up of infected malaria patients. It was our aim to evaluate the CellaVision ARBCA for screening and follow-up of malaria infections.

2. Materials and methods

For this study, 40 peripheral blood samples (31 positive and 9 negative samples) were collected in EDTA-anticoagulated tubes. The positive samples contained parasites of *P. falciparum* ($n = 21$), *P. malariae* ($n = 3$), *P. ovale* ($n = 4$) and *P. vivax* ($n = 3$). Twenty-three samples were obtained from the reference laboratory at the Institute of Tropical Medicine (ITM, Antwerp, Belgium). The remaining 8 positive samples were collected in the AZ Sint-Jan Hospital (Bruges, Belgium) and referred to the ITM for confirmation. The study was performed in a region devoid of endemic plasmodium prevalence and all positive samples were drawn from patients presenting with fever of unknown origin who resided in a region with a possible risk of malaria infection. Negative samples were selected from the routine hematology laboratory of AZ Sint-Jan and originated from hospitalized or ambulant patients with pathologies other than a suspected malaria infection.

All samples were characterized as described previously (Gillet et al., 2009) and anonymized. For this study, a thin smear was prepared, stained (May-Grünwald-Giemsa) and analyzed microscopically (Leica Microsystem, Wetzlar, Germany) on 1000× magnification (used as reference method) and digitally with CellaVision by 3 different trained operators. No additional information regarding patient history or other diagnostic tests was available for the operators.

For each sample, the mean parasitemia, obtained from microscopic evaluation from the 3 operators, was calculated and was used as gold standard. CellaVision ARBCA (software version 5.0.1) pre-reclassification as well as post-reclassification percentages of infected RBC were compared with this gold standard. Sensitivity and specificity were determined by receiver operating characteristic (ROC) curve analysis. Method comparison was performed using Passing-Bablok regression and Pearson's correlation coefficient. Method comparison and ROC analysis were carried out for all samples in total and for *P. falciparum* positive samples only. Indeed, we hypothesized that results for CellaVision would be better when only *P. falciparum* positive samples were analyzed as this parasite mostly presents as ring forms which can be more easily detected by the software compared to the more complex schizont or gametocyte forms of *P. vivax*, *P. ovale* and *P. malariae*.

Within-run variability was assessed by analyzing one slide of three peripheral blood samples with different parasite densities (negative sample, 0.15% and 3.43%) in 10 sequential runs on CellaVision. Between-run variability was evaluated by measuring the same 3 samples once daily on 10 different days. Coefficients of variation (CV) were calculated for both pre- and post-reclassification results. For estimation of inter-observer variability, 5 different observers trained for malaria microscopy each analyzed 5 different samples (negative, parasitemia 0.08%, 0.32%, 1.47% and 17.27%) manually as well as on CellaVision and mean, standard deviation (SD) and CV were calculated.

Statistical analysis was performed using Microsoft® Excel 2010 (Microsoft Corp., Redmond, WA, USA) and MedCalc® version 12 (MedCalc Software bvba, Mariakerke, Belgium).

3. Results and discussion

For a cut-off value of >0,0 for pre-reclassification results, ROC analysis (Table 1) yielded a sensitivity and specificity for detecting malaria parasites of 72% and 91% respectively. For post-reclassification, with an optimal cut-off of 0, sensitivity remained 72%, but specificity decreased to 82%, due to one additional false positive result. When only *P. falciparum* samples were included, sensitivity increased to 90% for

Table 1
Passing-Bablok regression analysis, Pearson's correlation coefficient and ROC curve analysis for CellaVision pre- and post-reclassification results compared to gold standard microscopy.

	Sample type	Intercept (95% CI)	Slope (95% CI)	R	ROC curve analysis		
					AUC (95% CI)	Sensitivity(%)	Specificity(%)
Microscopy – pre-reclassification	All samples	0.00 (0.00–0.00)	0.75 (0.56–0.91)*	0.94 ($p < 0.0001$)	0.84 (0.69–0.94)	72	91
	P. falciparum positive samples	0.00 (−0.09–0.01)	0.78 (0.67–1.16)	0.93 ($p < 0.0001$)	0.95 (0.75–1.00)	90	NA
Microscopy – post-reclassification	All samples	0.00 (−0.01–0.00)	0.71 (0.67–0.85)*	0.99 ($p < 0.0001$)	0.81 (0.66–0.92)	72	82
	P. falciparum positive samples	0.00 (−0.05–0.04)	0.74 (0.70–1.06)	0.99 ($p < 0.0001$)	0.97 (0.80–1.00)	95	NA
Pre-reclassification – post-reclassification	All samples	0.00 (0.00–0.00)	1.00 (1.00–1.19)	0.96 ($p < 0.0001$)	/	/	/

Analysis was carried out for all samples ($n = 40$) and *for P. falciparum* positive samples only ($n = 21$). Additionally, CellaVision pre-reclassification results were compared to post-reclassification results. NA: not assessed.
* Indicates a statistically significant result.

pre-reclassification and 95% for post-reclassification. None of the positive samples with a parasitemia <0.14% ($n = 5$) were detected by CellaVision. Logically, the detection rate of CellaVision increased with the parasite concentration and no false negatives were observed for parasite densities >0.37%, thereby covering hyperparasitemia as defined by the WHO (>2%) (World Health Organization, 2015).

Different reasons apply for the rather low sensitivity of the CellaVision RBCA for detection of malaria parasites. Firstly, percentages of parasitized RBC on CellaVision are displayed with only one decimal and percentages smaller than 0.05% are rounded down on CellaVision and reported as 0.0%, even when one or two infected RBC are recognized by the software and are presented to the operator. This is an important drawback as a considerable part of malaria infections present with a low parasitemia (<0.10%). When these samples were included as positive in sensitivity calculation, sensitivity increased from 72% to 79%. Secondly, CellaVision counts and photographs merely 1500–3000 RBC, which is considerably less than the amount of RBC screened during manual microscopy (usually around 10,000 RBC). Thirdly, only infected RBC in the trophozoite stage are recognized as RBC. RBC infected with schizonts or gametocytes are not recognized by CellaVision software, but may be classified as white blood cells, giant thrombocytes, thrombocyte aggregations, smudge cells or artifacts. However, these *Plasmodium* forms can be recognized by the operator when reviewing the white blood cells. Adequate parasitemia calculations are in these cases not

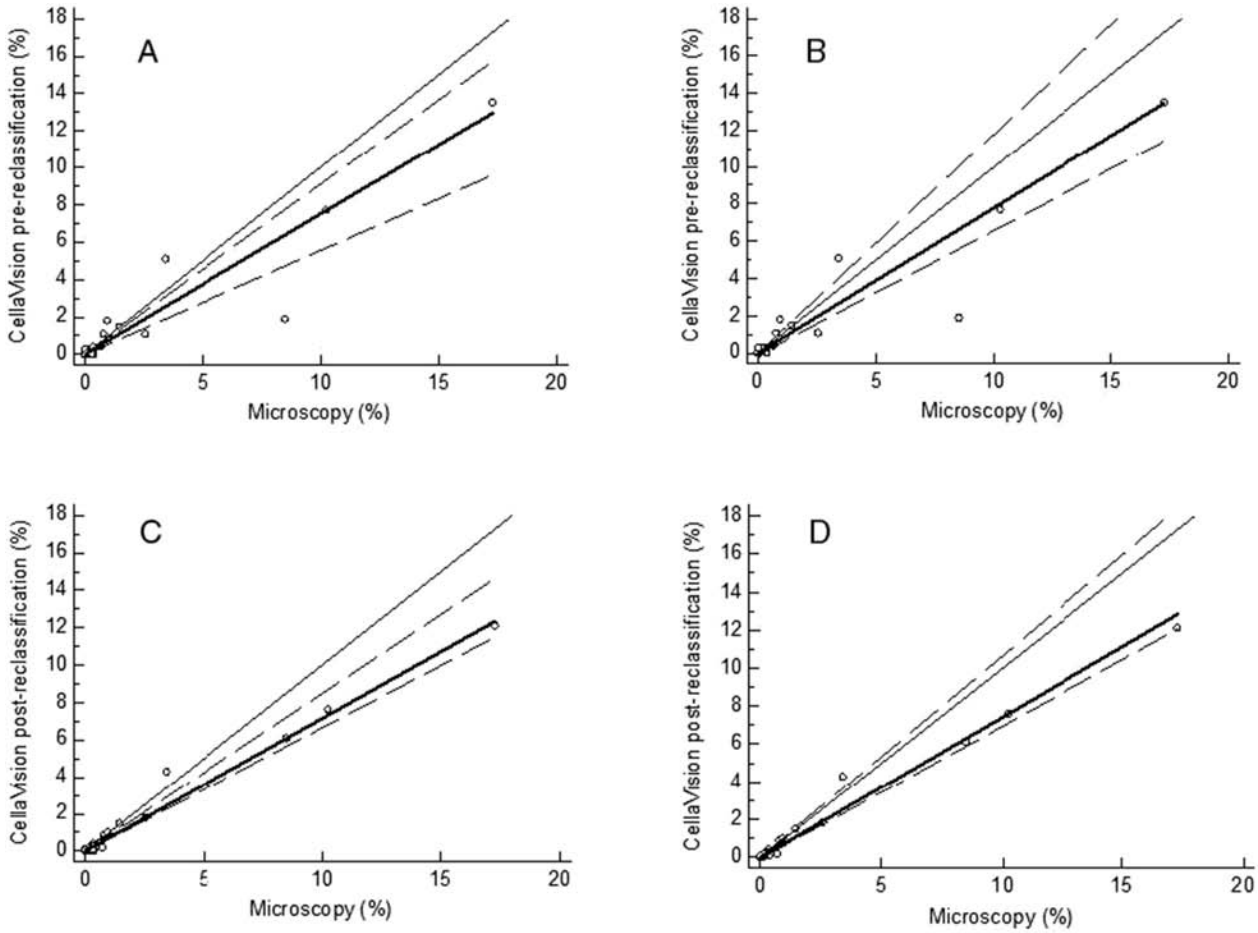

Fig. 2. Passing-Bablok regression analysis for CellaVision pre-reclassification compared to microscopy for all samples (A) and *P. falciparum* positive samples only (B). Passing-Bablok regression analysis for CellaVision post-reclassification compared to microscopy for all samples (C) and *P. falciparum* positive samples only (D). The thin solid line indicates the identity line (y = x). The bold solid line and the dashed lines represent the regression line and their 95% confidence interval respectively.

Table 2
Within-run and between-run variability.

	CellaVision pre-reclassification						CellaVision post-reclassification					
	Negative		Low		High		Negative		Low		High	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Within-run	0.08	49.0	0.33	24.5	6.1	9.4	0.0	/	0.10	0.0	3.9	11.5
Between-run	0.06	93.5	0.33	33.9	5.43	8.3	0.0	/	0.11	33.1	3.67	11.1

CV = coefficient of variation, expressed as %.

possible. A fourth limitation of the CellaVision ARBCA software is the rather low resolution of the RBC photographs. It is not always obvious for the laboratory technologist to distinguish malaria parasites from (giant) thrombocytes or artifacts such as air bubbles within the RBC. This limited resolution also does not allow to identify *Plasmodium* to the species level, which is crucial for therapy.

Passing-Bablok regression analysis results are shown in Table 1. Results are similar for both pre- and post-reclassification; both show a statistically significant proportional bias compared to microscopy, with lower values for CellaVision, especially in the high results range (Fig. 2A and B). Pearson's correlation coefficient was good for both methods (Table 1). When only *P. falciparum* positive samples are included in method comparison, a significant proportional bias was no longer observed, indicating that results between CellaVision (for pre- as well as post-reclassification) and manual microscopy are comparable although parasitemia results remained lower on CellaVision (Fig. 2C and D). Passing-Bablok regression between pre-reclassification and post-reclassification results showed no significant differences. However, during this evaluation it was observed that several false positive and false negative RBC had to be reclassified by the operator, often coincidentally resulting in similar final parasitemia between pre- and post-reclassification, despite numerous reclassifications within the same sample.

The inferior sensitivity compared to microscopy renders the CellaVision ARBCA unsatisfactory as a screening tool for malaria diagnosis. However, results correlate well with those of microscopic parasitemia evaluation. Thus, once a diagnosis has been made with other techniques (thick smear, rapid antigen test), the CellaVision could be used in the initial evaluation and follow-up of malaria parasitemia.

A previous study evaluated the performance of the CellaVision DM96 software, without the Advanced RBC Application, for qualitative detection of malaria and found detection rates similar to routine microscopy (Racsa et al., 2015). In earlier work of our group, the CellaVision ARBCA was evaluated for all RBC categories, including infection with malaria parasites (Criel et al., 2016). Results were considered unsatisfactory since, for a cut-off of ≥0.1, sensitivity was 40% and specificity was 78%. When a cut-off of 0 was used, sensitivity and specificity were respectively 100% and 0%. However, these results should be interpreted with caution since only 5 samples were included.

Within- and between-run variabilities for post-reclassification were lower or comparable to pre-reclassification and in general considered acceptable (Table 2). For inter-observer variability (Table 3), we could not demonstrate that variability was lower for CellaVision than for microscopy. High CVs for the negative and low positive samples were the statistical result of low mean values.

Strengths of the system are the presentation of infected RBC as individual cells which enhances the possibility of discussion amongst colleagues in case of doubt and which makes the CellaVision an excellent teaching tool. Moreover, the CellaVision gives an exact count of infected RBC on a precise number of total RBC, while the counting of total number of RBC per field by microscopy is a subjective estimation and not standardized between observers. Furthermore, since the ARBCA software is fast and easy-to-use, turn-around-times for parasitemia evaluation are shorter for CellaVision compared to microscopy.

A drawback of this study is the small sample size. Further studies, possibly with larger sample sizes, are required to confirm our findings regarding the performance of CellaVision ARBCA for screening and follow-up of malaria parasitemia. Additionally, only diagnostic samples were included in this evaluation. Follow-up samples from patients positive for malaria or samples from patients receiving therapy were not analyzed. Treatment may influence parasitemia morphology, therefore the performance of CellaVision ARBCA for detection of malaria parasites after therapy needs to be further evaluated.

We conclude that, despite the limitations of the CellaVision ARBCA for diagnostic screening of malaria parasites, it may still be helpful in routine practice for follow-up of known positive cases. The CellaVision ARBCA is a valuable, reproducible and fast alternative to manual microscopy for follow-up of parasitemia. Further studies may be needed to confirm these findings.

Table 3
Inter-observer variability.

	Microscopy			CellaVision post-reclassification		
Sample no.	Mean	SD	CV(%)	Mean	SD	CV(%)
1	13.47	0.85	6.3	12.96	0.65	5.0
2	0.38	0.08	21.5	0.38	0.08	22.0
3	0.02	0.02	91.3	0.04	0.05	136.9
4	0.00	0.00	/	0.00	0.00	/
5	1.57	0.55	35.2	1.54	0.09	5.8

SD = standard deviation; CV = coefficient of variation, expressed as %.

Acknowledgments

The authors declare no conflict of interest.

The authors would like to thank Sara Van Reeth and Tine Lowyck for their skillful morphological analyses.

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