Research Article   **Open Access**

An Enhanced Computer Vision Platform for Clinical Diagnosis of Malaria

Arnon Houri-Yafin[1], Yochay Eshel[1], Natalie Lezmy[1], Benedicta Larbi[2], Emma Wypkema[2], Veena Dayanand[3], Sarah Levy-Schreier[1], Caitlin Lee Cohen[1], Joseph Joel Pollak[1] and Seth J. Salpeter[1*]

[1]Sight Diagnostics Ltd., Israel

[2]Department of Clinical Hematology Lancet Laboratories, Lancet Corner, South Africa

[3]Department of Parisitology, City Hospital Mangalore, India

*Corresponding author: Seth Salpeter, Sight Diagnostics Ltd., Jerusalem Technology Park, Jerusalem 96951, Israel, Tel: 972-2-673-7370; E-mail: seth@sightdx.com

Abstract

Accurate malaria diagnosis is necessary to prevent unnecessary deaths and curb malaria drug resistance related to unnecessary treatment. While numerous diagnostic assays exist, the need for a low-cost, rapid and highly accurate malaria test remains. Here we evaluate the diagnostic performance of a computer vision platform, the Sight Diagnostic P2 device for malaria diagnosis, speciation and parasite quantification. The trial was conducted at two centers on *Plasmodium falciparum* and *Plasmodium vivax* samples, using different testing protocols: 374 samples were collected at City Hospital Mangalore India and 167 samples were collected at Lancet Laboratories Johannesburg South Africa. At City Hospital, the device diagnoses were compared to RT-PCR results while at Lancet Laboratories the device diagnoses were compared to a panel of tests provided by the clinic. For identification of malaria, the device demonstrated a sensitivity of 97% and a specificity of 99.5% at City Hospital India, and a sensitivity of 97.8% and a specificity of 97.5% at Lancet Laboratories Johannesburg. For speciation, the device correctly identified 87.5% for *Plasmodium Vivax* and 93.5% for *Plasmodium Falciparum* at City Hospital India. Lastly, comparing the device parasite count with that of trained microscopes, produced an average pearsons correlation of 0.87.

Keywords: Malaria; Diagnostic; Computer vision; Machine Learning

Abbreviations:

Pf: *Plasmodium falciparum*; Pv: *Plasmodium vivax*; Po: *Plasmodium ovale*; Pm: *Plasmodium malarie*

Introduction

Accurate diagnosis of malaria is imperative to reduce morbidity, prevent resistance to anti-malarials, and limit the number of adverse treatment effects from unnecessary use [1]. Furthermore, many studies show that infectious malaria carriers maintain a very low parasitemia, making sensitive detection technologies imperative for treatment targeted at epidemiologic control [2,3]. Many governmental health organizations now require patients to undergo malaria testing before receiving any anti-malarial medicine. As a result, the WHO forecasts an increase in global demand for malaria tests from 500 million in 2012 to nearly 1 billion tests by 2020 [1,4].

Due to increased demand for malaria tests, reliable, simple and highly accurate malaria diagnostic is needed. Globally, only 77% of suspected cases in the public sector are tested, while in Africa only 47% of cases are assayed [5]. A recent report showed that in some regions 81% of people taking ACT therapy are not infected, while only 31% of the positive cases received the treatment [6]. This glaring disparity not only leaves the needy untreated but encourages the further development of drug resistant malaria strains.

While new diagnostic modalities for malaria have emerged in recent years, none have the ideal set of test characteristics. According to the World Health Organization, an ideal test would be inexpensive, consistent, highly-sensitive, adequately specific, quantitative, and species-differentiating. Microscopy remains the gold standard malaria test worldwide [7,8], as it supports direct parasite identification and also provides monitoring of systemic inflammation and its response to therapy [9]. However, microscopy can be very inaccurate, needs extensive analysis, and requires highly trained staff [10,11]. Notably, malaria is associated with systemic spiraling of innate inflammation and additional blood abnormalities, further complicating microscopy examination [12]. Numerous reports have also shown inconsistent sensitivity of microscopist due to the high volume of tests and varied level of skill among malaria technicians [13,14]. Rapid diagnostics tests (RDTs) continue to increase in popularity and market share as they have significantly improved the diagnosis of malaria in remote, inaccessible areas [15]. However, RDTs have significant limitations that make many practitioners wary of their use, including decreased sensitivity at low parasitemia, inability to quantitate parasite burden, and inconsistencies between brands in their ability to detect and differentiate different malaria species [16,17]. Recent improvements to malaria diagnostic technologies include Polymerase Chain Reaction (PCR) and loop-mediated isothermal amplification (LAMP), which offer superior sensitivity, speciation and parasitemia but are impractical for the vast majority malaria-endemic areas [18,19].

Malaria diagnosis using computer vision offers a potential solution to the shortcomings of other technologies. An automated microscopist maintains the advantages of a microscopist with significant improvements in speed, cost, and consistency. Previous attempts at a creating a computer microscopist have not surpassed the development stage [20,21]. A recent report from our group, described a clinically available automated microscopist which was tested with the National Institute of Malaria Research India [22]. The device, the SightDx P1

malaria platform, was tested on 431 patients and demonstrated a sensitivity of 97.05%, and a specificity of 96.33% when compared with PCR. Furthermore, the device was able to accurately speciate 73.3% of the PCR *Plasmodium falciparum* and 91.4% of the PCR *Plasmodium vivax* samples, and showed a parasitemia correlation with microscopists of 0.89.

Here, we present an enhanced version of the Sight Diagnostic malaria device, the SightDx P2 platform for malaria detection. The device is intended for laboratories performing high volumes of malaria tests as it is capable of scanning a sample in 4 minutes and can hold up to 30 tests. The system has dimensions of 45 x 50 x 58 cm (DxWxH) and can easily fit onto a standard laboratory bench top with minimal installation requirements.

In the following report, we describe the results of clinical studies performed in Mangalore India, and Johannesburg South Africa to evaluate the sensitivity, specificity, speciation and parasite count calculation as compared to standard diagnostic procedures.

Methods

Study design

The study was a double center, prospective, non-randomized, non-blinded study conducted at City Hospital, Mangalore India with 374 blood samples from clinically-suspected malaria patients, and at Lancet Laboratories Johannesburg South Africa on 167 clinically suspected malaria patients.

Study Procedures

City Hospital, Mangalore India: Determination of eligibility for malaria treatment was solely based on the clinic's standard diagnosis protocol and the patients course of treatment and was not altered due to the study or the SightDx diagnostic device.

In most cases blood was scanned by the device within 24 hours of sampling. Samples more than 48 hours old were not included in the study. In addition, 100 µL of blood was collected on GE Healthcare FTA Whatman filter paper spots for RT-PCR evaluation. RT-PCR results were considered the standard of comparison for determining the sensitivity, specificity and speciation of the various methods.

Lancet Laboratories, Johannesburg South Africa: Samples were provided from malaria tests performed at Lancet Laboratories Johannesburg and at surrounding Lancet Laboratory clinics in South Africa. Samples were tested on the Sight Diagnostic device within 1 week of drawing. RDT and microscopy were performed on all samples. Discrepancies between these tests were evaluated by PCR. Positive samples which were not *Plasmodium falciparum* or had a parasitemia under 1000 parasites/µL also underwent PCR. All negative samples were reviewed with QBC.

Laboratory Methods

Sight Diagnostic Device Analysis

In all locations digital imaging scanning was carried out onsite. To begin sample diagnosis, 5 µL of patient blood was mixed with a fluorescent dye solution that stained intracellular DNA and RNA. The sample was then loaded into a plastic cartridge and incubated for 5 minutes, during which time the cells formed a monolayer. The stained cells were then excited using 3 different LED light sources (370 nm, 475 nm and 530 nm) after which the imaging system recorded 600 images analyzing ~1.8 million cells. The total scan time per sample was 4 minutes and the device held up to 30 samples which can be loaded in batch. Samples which registered an error on the device due to incorrect user preparation were repeated. Computer vision and statistical models were used to detect the malaria parasites. Using statistical models, the device determined infection status, parasitemia levels, and species.

Parasitemia

Parasitemia counts were performed on 24 positive samples at Lancet laboratories Johannesburg. An expert microscopist analyzed 10 fields at 100X with approximately 100 RBCs counted per field. Parasitemia was calculated as a ratio of infected RBCs to total RBCs.

Real Time PCR Analysis

For PCR experiments performed on samples from City Hospital India, a whole punch was removed from the blood spot on the GE FTA Whatman paper and eluted as previously reported [23]. Real time PCR was performed with Fast Syber Green Master Mix at a volume of 10 µL (Applied Biosystems) using previously published primer sequences [24] for identifying *falciparum, vivax* and for general Plasmodium (Plu). All reactions were performed in 384 well qPCR plates (Bio-Rad) on a CFX384 real time PCR machine from Bio-rad.

Results

The Sight Diagnostic P2 malaria scanning device is a desktop system for computerized malaria diagnostics (Figures 1A and 1B). The stained blood is loaded into a cartridge which holds five patients samples. To evaluate device performance in a clinical setting, 374 samples were collected and scanned at City Hospital Mangalore and 167 samples were collected and scanned at Lancet Laboratories Johannesburg.
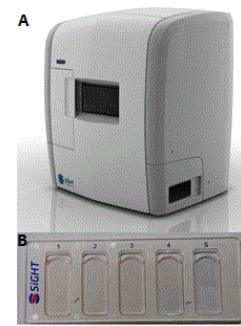
Figure 1: The SightDx Malaria Platform. (A) The P2 malaria scanning device. (B) The loading cartridge holds 5 patient samples.

Sensitivity and specificity

Sensitivity and specificity were analyzed for all trials (Table 1). For samples scanned at City Hospital India device results were compared to qPCR while for samples scanned at Lancet Johannesburg device results were compared to a final diagnosis based on a combination of several malaria diagnostic assays (Table 1). At City Hospital Mangalore

sensitivity was calculated as 97% (167/172) and specificity was calculated as 99.5% (201/202). For samples scanned at Lancet Laboratories Johannesburg sensitivity was 97.8% (46/47) while specificity was 97.5% (117/120). Positive predictive values (PPV) were 99.4% at City Hospital and 93.8% at Lancet Laboratories, and negative predictive values (NPV) were 97.5% at City Hospital and 99.1% at Lancet Laboratories.

	Sensitivity			Specificity		
	Percent	Ratio	95% CI	Percent	Ratio	95% CI
City Hospital India	97%	167/172	0.934-0.988	99.50%	201/202	0.972-0.999
Lancet Labs South Africa	97.80%	46/47	0.843-0.994	97.50%	117/120	0.929-0.991

Table 1: Sensitivity and specificity of device vs. ground truth. Sensitivity and specificity are presented as individual numbers for both trial sites.

Speciation

Speciation studies were conducted on samples provided at City Hospital Mangalore (Table 2). At City Hospital, the device distinguished between P.v (*Plasmodium vivax*) and P.f (*Plasmodium falciparum*) and results were compared to qPCR analysis. A total of 167 samples were identified as positive by the device and were analyzed for species type. The device correctly identified samples with *Plasmodium vivax* at 87.5% sensitivity (119/136) and *Plasmodium falciparum* at 93.5% sensitivity (29/31).

	City Hospital (India)		
	Percent	Ratio	95% CI
Plasmodium Vivax	87.50%	119/136	0.809-0.92
Plasmodium Falciparum	93.50%	29/31	0.793-0.982

Table 2: Speciation accuracy divided according to treatment groups. Speciation percentages of the trials from City Hospital India are presented in the table, as well as the specific number of patients and confidence Index.

Parasitemia

For cases diagnosed at Lancet Laboratories with thin smear microscopy, parasitemia was provided and compared to values from the device (Figure 2).
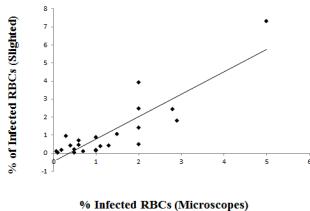
Figure 2: Micrscopist compared to the device. At Lancet Laboratories a trained microscopist analyzed 24 slides and the results were compared to the parasitemia reported by the device. The correlation between the two produced a Pearsons correlation coefficient of 0.87.

A comparison of the percentage of infected RBC determined by the microscopist and the device yields a Pearsons correlation coefficient of 0.87. The microscopist calculated parasitemia by analyzing the number of infected red blood cells out of the total number of blood cells.

Discussion

This study evaluated the SightDx P2 malaria detection platform, an enhanced computer vision platform for rapid and automated malaria diagnostics. Previous attempts to develop vision based malaria detection devices have had varying levels of success [21,25-28]. While a specific report showed high sensitivity and specificity [29], others demonstrated relatively low performance numbers. Notably, these papers describe development stage technologies showing initial device construction or preliminary algorithm designs for malaria detection.

Previous studies showed problems in cartridge design and focus mechanisms, yielding slow scanning times and poor results. While these reports used complicated microfluidics systems, our study presents an easy to use plastic cartridge which fills quickly upon loading using capillary forces activated by mixing the blood with our stain solution. Moreover, we have solved image focus difficulties, by implementing unique algorithms which allow the scanning system to quickly autofocus on each new field allowing for high quality images of all cells scanned.

In a previous study [22] we presented the first clinically available computer vision based reader for malaria diagnostics. The P1 device showed a sensitivity of 97.05%, specificity of 96.33% and speciation of 73.3% *Plasmodium falciparum* and 91.4% for *Plasmodium vivax*. The P2 device features many functional and performance based improvements over the earlier system. While the P1 device holds only 5 patient samples, the P2 machine holds 30 samples and is capable of asynchronous batch loading. Moreover, the P1 device requires 8 minutes to scan a sample while the P2 device requires only 4 minutes, allowing for the rapid scanning of large volumes of specimens. On a performance level, the device showed a similar sensitivity at an average of 97.4% but a significantly improved specificity at an average of 98.5%. Speciation of *Plasmodium vivax* was comparable to the previous study at 87.5% while speciation of *Plasmodium falciparum* was significantly improved at 93.5%.

Importantly, in contrast to the study on P1 which was performed only in India, the current trial was conducted on samples from both continental Africa and India. Numerous studies have shown the ability of strains of malaria to develop mutations causing significant difficulties in diagnosis [1,30,31]. In particular, it has been shown that RDTs which identify HRP-2 from *Plasmodium falciparum* can yield false negatives due to specific antigen mutations [32]. Specific regions are known to develop unique genetic variants of even the most

standard species of Plasmodium. Our results confirm the devices ability to detect strains of malaria in variety of geographical regions.

In the current study, the device maintains a limit of detection of 50 parasites/ µL. While the system is capable of identifying as few as 5 parasites/ µL the current algorithm only identifies a positive sample if it detects more than 50 objects identified as malaria parasites. This limitation was evident from the six cases of false negatives where the parasitemia was found to be under 50 parasites/ µL, explaining the misdiagnoses. Decreasing the current limit of detection causes an increase of false positives reported by the system. False positives have been found to be caused by particles that have flourescence morphology similar to the stained malaria within the RBC. The four false positives found in our study were determined to result from Howell Jolly bodies which are malaria-like DNA/RNA fragments found in RBCs. By collecting larger libraries of samples, as well as samples with Howell Jolly bodies, we will be able to apply machine learning to improve the accuracy of the algorithm classification and overall diagnosis. Additional data collection and algorithm design work will be necessary to further improve the differentiation between malaria and these objects to lower the limit of detection.

Several device improvements are currently under development to strengthen diagnostic performance and provide additional clinical information to assist in patient treatment. Speciation for P.v was calculated at 87.5% and P.f at 93.5%, leaving room for increased accuracy. As the device speciation is based on a machine learning algorithm which improves with an increased database, the collection of additional scanned samples of both P.v and P.f should significantly improve speciation results. Moreover, to expand speciation capabilities to *Plasmodium ovale*, *Plasmodium malarie* and mixed infection a large library of P.o and P.m and mixed infection samples will need to be collected and analyzed. Additional studies will also be necessary which feature completely blinded data collection as well as PCR ground truth for all samples.

References

1. Unitaid V (2014) Malaria Diagnostics Technology and Market Landscape (2nd Edn).
2. Dal-Bianco MP, Köster KB, Kombila UD (2007) High prevalence of asymptomatic Plasmodium falciparum infection in Gabonese adults. Am J Trop Med Hyg 77: 939-942.
3. Klein EY, Smith DL, Boni MF, Laxminarayan R (2008) Clinically immune hosts as a refuge for drug-sensitive malaria parasites. Malar J 7: 67.
4. Kiszewski A, Johns B, Schapira A (2007) Estimated global resources needed to attain international malaria control goals. Bull World Health Organ 85: 623-630.
5. ACTwatch.
6. Cohen JM, Woolsey AM, Sabot OJ, Gething PW, Tatem AJ, et al. (2012) Public health. Optimizing investments in malaria treatment and diagnosis. Science 338: 612-614.
7. Yan J, Li N, Wei X (2013) Performance of two rapid diagnostic tests for malaria diagnosis at the China-Myanmar border area. Malar J 12: 73.
8. Arya SC, Agarwal N (2013) Laboratory tests for malaria: a diagnostic conundrum. S Afr Med J 103: 701.
9. (2012) Relevance Of Economic Field Microscope In Remote Rural Regions For Concurrent Observation Of Malaria & Inflammation, Advances In Infectious Diseases (AID).
10. Harchut K, Standley C, Dobson A (2013) Over-diagnosis of malaria by microscopy in the Kilombero Valley, Southern Tanzania: an evaluation of the utility and cost-effectiveness of rapid diagnostic tests. Malar J 12: 159.
11. Fançony C, Sebastião YV, Pires JE, Gamboa D, Nery SV (2013) Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. Malar J 12: 284.
12. Bhattacharya D, Bhuyan BM, Pradhan PK, Nayak DK (2013) Transmission Blocking of Year Round Resistant Malaria in Koraput (India) by OMARIA– A New Antimalarial Phytotherapy. British Journal of Pharmaceutical Research 3: 54-77.
13. Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, et al. (2007) Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial. BMJ 334: 403.
14. Ansah EK, Narh-Bana S, Epokor M (2010) Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: a randomised controlled trial in Ghana. BMJ 340: c930.
15. Kumar A, Chery L, Biswas C, Dubhashi N, Dutta P, et al. (2012) Malaria in South Asia: prevalence and control. Acta Trop 121: 246-255.
16. Mouatcho JC, Goldring JP (2013) Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol 62: 1491-1505.
17. Alonso PL, Tanner M (2013) Public health challenges and prospects for malaria control and elimination. Nat Med 19: 150-155.
18. Hopkins H, González IJ, Polley SD (2013) Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. J Infect Dis 208: 645-652.
19. Coleman RE, Sattabongkot J, Promstaporm S, Maneechai N, Tippayachai B, et al. (2006) Comparison of PCR and microscopy for the detection of asymptomatic malaria in a Plasmodium falciparum/vivax endemic area in Thailand. Malar J 5: 121.
20. Kaewkamnerd S, Uthaipibull C, Intarapanich A, Pannarut M, Chaotheing S, et al. (2012) An automatic device for detection and classification of malaria parasite species in thick blood film. BMC Bioinformatics 17: S18.
21. Vink JP, Laubscher M, Vlutters R, Silamut K, Maude RJ, et al. (2013) An automatic vision-based malaria diagnosis system. J Microsc 250: 166-178.
22. Srivastava B, Anvikar AR, Ghosh SK (2015) Computer-vision-based technology for fast, accurate and cost effective diagnosis of malaria. Malar J. 14: 526.
23. Bereczky S, Mårtensson A, Gil JP, Färnert A (2005) Short report: Rapid DNA extraction from archive blood spots on filter paper for genotyping of Plasmodium falciparum. Am J Trop Med Hyg 72: 249-251.
24. Kamau E, Alemayehu S, Feghali KC, Saunders D, Ockenhouse CF (2013) Multiplex qPCR for detection and absolute quantification of malaria. PLoS One 8: e71539.
25. Xie L, Li Q, Johnson J, Zhang J, Milhous W, et al. (2007) Development and validation of flow cytometric measurement for parasitaemia using autofluorescence and YOYO-1 in rodent malaria. Parasitology 134: 1151-1162.
26. Prescott WR, Jordan RG, Grobusch MP, Chinchilli VM, Kleinschmidt I, et al. (2012) Performance of a malaria microscopy image analysis slide reading device. Malar J 11: 155.
27. Frean JA (2009) Reliable enumeration of malaria parasites in thick blood films using digital image analysis 8: 218.
28. Purwar Y, Shah SL, Clarke G, Almugairi A, Muehlenbachs A (2011) Automated and unsupervised detection of malarial parasites in microscopic images. Malar J 10: 364.
29. May ZAS, Salamat R (2013) Automated quantification and classification of malaria parasites in thin blood smears. Signal and Image Processing Applications (ICSIPA), IEEE International Conference 8-10.
30. Atroosh WM, Al-Mekhlafi HM, Mahdy MA, Surin J (2012) The detection of pfcrt and pfmdr1 point mutations as molecular markers of chloroquine drug resistance, Pahang, Malaysia. Malar J 11: 251.
31. Pillai DR, Lau R, Khairnar K (2012) Artemether resistance in vitro is linked to mutations in PfATP6 that also interact with mutations in

PfMDR1 in travellers returning with Plasmodium falciparum infections. Malar J 11: 131.

32. Kumar N, Singh JP, Pande V (2012) Genetic variation in histidine rich proteins among Indian Plasmodium falciparum population: possible cause of variable sensitivity of malaria rapid diagnostic tests. Malar J 11: 298.