APDS (AUTOMATED PARASITE DETECTION SYSTEM) FOR FIELD MALARIA DIAGNOSIS IN THE PHILIPPINES

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Abstract. Automated Parasite Detection System (APDS) is an enhancement of the reference standard conventional microscopy, intended to be developed into a low-cost tool to consistently read thin blood films using software for rapid diagnosis. A cross-sectional study design was applied in determining the accuracy of measures: sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), of APDS with conventional microscopy as reference standard using human thin blood films obtained from patients in Palawan, Philippines. A total of 267 images were collected, with 132 positives and 135 negatives. APDS exhibited 96.97% sensitivity and 91.11% specificity, in comparison with conventional microscopy. APDS also reported 2-75% parasitemia from the images analyzed, with a lowest count of one parasite per field, further emphasizing its sensitivity. Thus, APDS presents to be a potential screening tool for malaria field diagnosis.

Keywords: malaria, falciparum, microscopy, parasitemia, Philippines

INTRODUCTION

Malaria is one of the leading causes of deaths worldwide, as it is being transmitted in all six regions of the World Health Organization (WHO, 2014). *Plasmodium falciparum (Pf)* is the most harmful species to cause malaria in humans, with female *Anopheles* mosquitoes serving as vectors. Other species also known to infect humans are *P. vivax (Pv), P. ovale, P. malariae,* and *P. knowlesi.* About 3.3 billion people are at risk of acquiring malaria, as estimated by WHO in 2013, with 1.2 billion at high risk of getting malaria in a year (WHO, 2014). In the Philippines, reported malaria cases decreased by 75% and deaths lowered by 99% for the past 10 years, allowing the nation to being a step closer towards malaria elimination by 2030 (DOH, 2012, 2014). About 99% of the total of 8,086 malaria cases reported came from 15 out of 80 provinces in the Philippines reported in 2012, and among these provinces, the major contributors to these numbers are the provinces of Palawan (51%) and Tawi-Tawi (34%) (DOH, 2014). In due time, WHO aims for a malaria-free world through appropriate implementa-

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tion of measures, such as vector control, accurate diagnosis, and proper treatment administration, with a goal of reducing malaria cases globally by 75% in 2015. With the annual rate decreasing for the past 13 years and should it be maintained, it is projected that the mortality rates due to malaria will be decreased by 55% globally and 62% in the WHO African region (WHO, 2014).

To effectively control malaria, prompt malaria detection is critical to avoid misdiagnosis and inappropriate intake of antimalarial medicines. Conventional microscopy of thin blood films remains to be the reference standard in malaria diagnosis despite the development of other diagnostics (Laoboonchai *et al*, 2001; Paris *et al*, 2007; Karl *et al*, 2009; Muerhoff *et al*, 2010; Thiramanas *et al*, 2010; Taviad *et al*, 2011), due to its low cost and wide applicability in any location. However, examination time and detection limit are highly dependent on the ability of the microscopist (Tangpukdee *et al*, 2009).

This study aims to improve on conventional malaria microscopy using thin blood films by automating the technique to remove subjectivity due to technician expertise level. Automated microscopy can exhibit more consistent diagnosis, especially when examining large number of samples, while requiring less time than manual examination. Automated Parasite Detection System (APDS) is a novel detection system intended for malaria diagnosis. It consists of a program that rapidly detects and counts malaria parasites in microscopic images of Giemsa-stained thin blood films, reflecting the intensity of infection. APDS will be demonstrated and validated for malaria field diagnosis by comparing it with conventional Giemsa microscopy, in Palawan, Philippines, an area known to be malaria-endemic.

MATERIALS AND METHODS

Study design

A descriptive cross sectional study design was utilized in determining the accuracy of measures [sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV)] of APDS in malaria diagnosis with conventional microscopy as reference standard in viewing thin blood films. Performance of APDS was validated using human blood samples of volunteers manifesting clinical symptoms of malaria from Puerto Princesa, Palawan.

Study site

Blood samples were collected from the province of Palawan, specifically in Brgy. Luzviminda, San Rafael, and Puerto Princesa City Proper. The study site, known for being malaria-endemic, was chosen due to its high number of malaria cases in 2011, with the highest number of severe cases found in the municipality of Brooke's Point, followed by Puerto Princesa City, as reported by the Provincial Health Office of Palawan. *Plasmodium falciparum, P. vivax* and *P. malariae* infections were reported to be present in the study site.

Automated microscope system

The microscope system consists of a binocular microscope (OMAX MD827S30 Series: Gyeonggi-do, Korea) with a built-in digital camera attached to the eyepiece and to a computer. Images obtained using the microscope were viewed through the computer and were subjected to image analysis using the software developed by Labrecque *et al* (APDS Beta version 46.3: Culex Innovations, Toronto, Canada) to detect parasites in the RBCs and determine the parasitemia. Configuration of the APDS software was based on images of

confirmed malaria-positive films archived at the College of Public Health, University of the Philippines-Manila. About 10-20 microscopic field images per thin film were collected to train the software for every possible parasite appearance. Each cell in an image was analyzed by the software based on the cell diameter, color, intensity, hue and saturation of the pixels.

Microscopic examination using APDS

Blood films collected from the study site were prepared using standard WHO procedures (WHO, 2010). Images of the Giemsa-stained thin blood films were collected at 1,000x oil immersion using the microscope setup. A total of 267 images of blood films were collected, satisfying the calculated minimum sample size of 59 (Banoo *et al*, 2006). The images were initially filtered by APDS to reduce background noise that may affect the identification of the RBCs, followed by edge detection and thinning of the cell diameter to identify the RBCs individually and separate them from other cells and artefacts found in blood. APDS emphasized the cell edges to enclose all RBCs and select only those which correspond to the size of RBC in terms of pixels (Fig 1).

After cell detection by size, RBCs were searched internally for the specific hue of dye identified by the software through an algorithm, by making use of a color histogram that plots all possible color pixels and selects the appropriate purple color of a malaria parasite with respect to the background color. Upon parasite hue detection inside the RBCs, infected cells were identified and enclosed in red borders, whereas uninfected ones are enclosed in green (Fig 2).The software output reported the presence or absence of parasites and its density by counting the infected RBCs found out of the total number of cells, indicating the parasitemia.

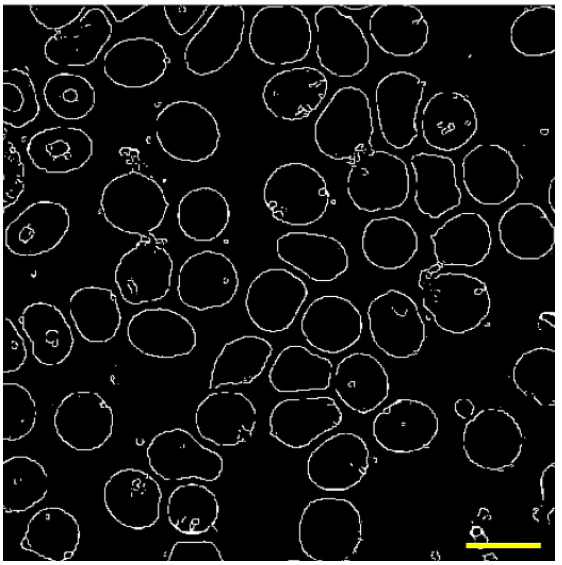

Fig 1–Edge and cell detection of RBCs, scale bar = 10 μm.

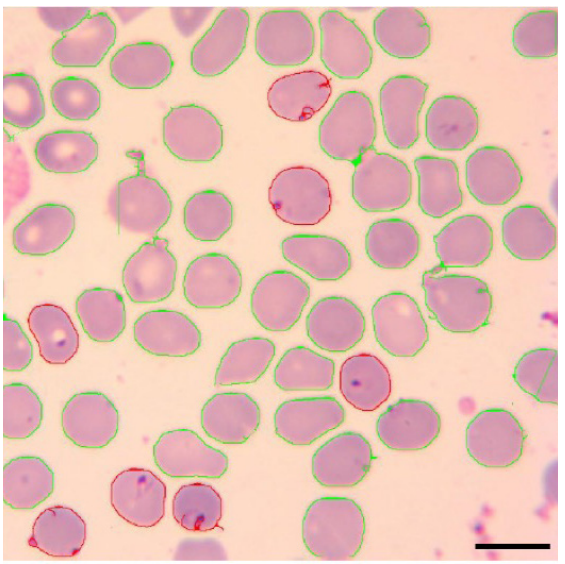

Fig 2–*P. falciparum* (ring form) infected (red) and uninfected cells (green) identified by APDS, scale bar = 10 μm.

Data analysis

The 95% Confidence intervals (CI) for sensitivity, specificity, PPV and NPV were calculated using OpenEpi v3. Parasitemia were calculated as percentage of infected

Table 1
APDS performance *vs* Giemsa microscopy in malaria parasite detection.

APDS	Microscopy (Reference standard)		Total
	Positive	Negative	
Positive	128	12	140
Negative	4	123	127
Total	132	135	267

Table 2
Validity of APDS *vs* Giemsa microscopy at 95% CI.

Parameter	Estimate (95% CI)
Sensitivity	96.97% (92.47-98.82)
Specificity	91.11% (85.11-94.84)
Positive Predictive Value (PPV)	91.43% (85.65-95.03)
Negative Predictive Value (NPV)	96.85% (92.18-98.77)

RBCs. The values were automatically reported by the software, and were arranged accordingly using Microsoft Excel to identify the frequencies of each reported percentage and describe the parasitemia levels of the samples.

Ethical considerations

The research protocol was reviewed and approved by the UP Manila Research Ethics Board. Approval from the Provincial Health Office of Palawan and City Health Office was obtained prior to conduct of the study. Participants were requested to sign an informed consent before performing any procedure.

RESULTS

Validity of APDS using conventional Giemsa microscopy as reference standard

A total of 267 images consisting of 132 true positives and 135 true negatives, as examined and confirmed by an expert microscopist, were obtained from the 21 thin blood films, which include 12 negative slides, 4 slides positive for *P. vivax*, and 5 slides positive for *P. falciparum*. Each image is treated as one sample given their variability in appearance and cell distribution in the microscopic field. The readings of APDS as compared to the microscopist diagnosis are summarized in Table 1.

Out of 132 positive images confirmed by conventional Giemsa microscopy, 128 images have been correctly identified as positive, while 4 images are falsely identified as negative by APDS. Parasite detection by APDS is regardless of species, so presence of either *Pf* or *Pv* or both are still identified as positive despite their difference in appearance. The 4 images detected as negative mostly included *P. vivax*, which exhibit irregularly-shaped RBCs that are slightly larger in size (Fig 3).

For the negative images, 123 images are properly identified as negative by APDS, while 12 images are identified as false positives. These false positives

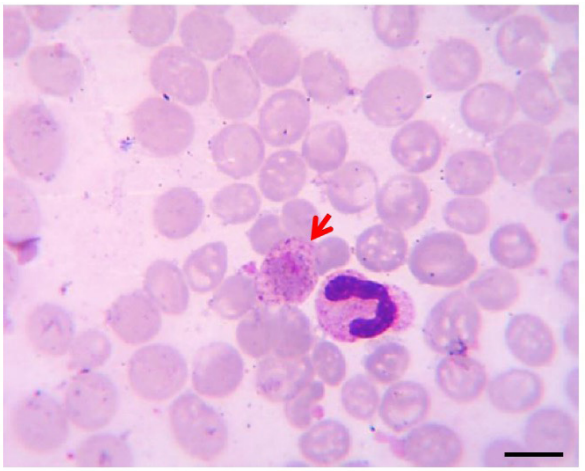

Fig 3–Thin blood film showing *P. vivax* trophozoite, scale bar = 10 μm.

have been observed to be mainly due to artifact staining found within the edges of an RBC similar to the color and shape of parasite, as those found outside are not considered as parasites by the software. Table 2 shows the summarized validity of APDS in comparison with the reference standard within 95% CI.

Parasitemia of malaria positive images

Parasite detection using APDS comes with a report of parasitemia in the form of infected cells out of total number of cells found in each image. Parasitemia levels are expressed as percentage (%) and frequency of the percentages are compiled to show the trend of parasitemia found in the blood films. Parasitemia levels ranging from 2% to 75% have been detected by APDS, whereas those with 3-31% showed to be more common. This shows that APDS is sensitive enough to detect as low as 2% parasitemia in an image. However, these values may not reflect the actual parasitemia levels of the entire blood film as APDS is restricted to the cells that have been focused in a certain image, and not all cells in a certain image can be in focus at the same time.

DISCUSSION

APDS goes through a series of steps to detect malaria parasite within the RBCs, but is limited to what has been focused by the microscope upon image capture. Images were captured from the feathered edge of the thin blood films to minimize overlapping of RBCs but then, thin films are still three-dimensional even if they appear as a monolayer of cells when viewed under the microscope, thus not all the RBCs are focused at the same time. Minimal adjustments of the microscope focus leads to differences in cell counts, however, the software performance remains unaffected. The software can analyze an image in about 40 seconds, and loop the program to read several images in one run, thus gathering readings in a uniform manner with real-time data collection and storage without its performance being affected by time. In this aspect, APDS exhibits better consistency in diagnosis, especially in reporting parasitemia, requiring lesser time than manually examining blood films.

Sensitivity of APDS (96.97%) shows to be higher than automated systems in the studies of Tek *et al* (2010) (72.4%) and Diaz *et al* (2009) (78.8%), but Purwar *et al* (2011) managed to develop a system that has 100% sensitivity. Comparing it to RDTs with a sensitivity of 95.3% for *Pf*, and 68.9% for *Pv* (Murray and Bennett, 2009), APDS has shown to have performed well enough, or even better, in identifying true positives.

APDS is found to have greater specificity (91.11%) than automated system in the study of Purwar *et al* (2011) (50-88%),

and comparable with Diaz *et al* (2010) (91.2%). This implies that APDS is able to identify true negative images with a probability of 91.11% compared to the reference standard, but still needs further improvement to be comparable to other current diagnostic tools such as RDTs, which are known to have specificity of 94.2% for *Pf* and 99.8% for *Pv* (Murray and Bennett, 2009).

Determining the parasitemia of malaria-infected blood films is important to know the severity of infection, to see how the patient responds to treatment, to be aware of the severity of cases in an area, and to monitor therapeutic efficacy (WHO, 2010). With the help of an automated system like APDS, monitoring malaria cases can be performed more rapidly and efficiently with human errors minimized, leading to more consistent data collection. Also, real-time surveillance of malaria cases can be achieved with a system such as APDS, making it easier for monitoring and reporting of cases, paving the way to a more efficient administration of treatment.

In conclusion, this study presented the potential of APDS as a novel malaria detection system, especially for rapid screening of thin blood films. APDS performed to have 96.97% sensitivity and 91.11% specificity, in comparison with conventional Giemsa microscopy. The software also reported the parasitemia of the thin films, demonstrating the ability of APDS to quantify detected parasites. Each image can be analyzed for 40 seconds, having an advantage of consistency in reading over time as compared to a microscopist, who can get tired through time. APDS is envisioned to become a useful tool in diagnostics and surveillance, especially when malaria cases and transmission become very low, making the detection of positive cases become more labor-intensive for microscopists. APDS can also be applied in mass screening of blood banks and diagnostic laboratories for high throughput analysis. With its current performance, the next step is to perform field testing of APDS to gather feedback from the microscopists themselves for further improvement of the system. In the future, APDS is aimed to be developed into a low-cost integrated device intended for field diagnosis especially in developing countries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this manuscript.

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