

## Evaluation of Malaria Diagnoses Using a Handheld Light Microscope in a Community-Based Setting in Rural Côte d'Ivoire

Jean T. Coulibaly,<sup>1,2,3,4</sup> Mamadou Ouattara,<sup>1,2</sup> Jennifer Keiser,<sup>3,4</sup> Bassirou Bonfoh,<sup>3,4</sup> Eliézer K. N'Goran,<sup>1,2</sup>  
Jason R. Andrews,<sup>5†</sup> and Isaac I. Bogoch<sup>6,7,8\*†</sup>

<sup>1</sup>Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire; <sup>2</sup>Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire; <sup>3</sup>Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland; <sup>4</sup>University of Basel, Basel, Switzerland; <sup>5</sup>Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA; <sup>6</sup>Division of General Internal Medicine, Toronto General Hospital, Toronto, Canada; <sup>7</sup>Division of Infectious Diseases, Toronto General Hospital, Toronto, Canada; <sup>8</sup>Department of Medicine, University of Toronto, Toronto, Canada

**Abstract.** Portable microscopy may facilitate quality diagnostic care in resource-constrained settings. We compared a handheld light microscope (Newton Nm1) with a mobile phone attachment to conventional light microscopy for the detection of *Plasmodium falciparum* in a cross-sectional study in rural Côte d'Ivoire. Single Giemsa-stained thick blood film from 223 individuals were prepared and read by local laboratory technicians on both microscopes under 1,000× magnification with oil. Of the 223 samples, 162 (72.6%) were *P. falciparum* positive, and the overall mean parasite count was 1,392/μL of blood. Sensitivity and specificity of the handheld microscope was 80.2% (95% confidence interval [CI]: 73.1–85.9%) and 100.0% (95% CI: 92.6–100.0%), respectively, with a positive and negative predictive value of 100.0% (95% CI: 96.4–100.0%) and 65.6% (95% CI: 54.9–74.9%), respectively. If sensitivity can be improved, handheld light microscopy may become a valuable public health tool for *P. falciparum* diagnosis.

### INTRODUCTION

The World Health Organization (WHO) announced a “T3: Test. Treat. Track” initiative on World Malaria Day in 2012, with the goal of scaling up diagnostic, treatment, and surveillance systems in malaria-endemic countries.<sup>1</sup> A central tenant of this initiative revolves around universal access to quality malaria diagnostics with both rapid diagnostic tests (RDTs) and microscopy.<sup>2</sup> The WHO recommends that all suspected cases of malaria be evaluated by either an RDT or microscopy before initiating antimalarial treatment. Ideally, the two diagnostic tests are meant to be used in a complementary manner, as each has benefits and limitations.<sup>1,3,4</sup> Malaria RDTs are designed for rapid, point-of-care malaria diagnoses; however, they are not ideal for quantifying parasitemia. These tests are designed to determine the presence or absence of infection and to dichotomize cases as either *Plasmodium falciparum* or a non-*P. falciparum* malaria species. Microscopy has the capabilities to speciate and quantify malaria diagnoses, but issues surround the general lack of availability, poor maintenance of microscopes, and training of microscopists in malaria-endemic zones.<sup>3,5–7</sup>

Microscopes are invaluable tools in clinical and public health settings. For example, in areas holoendemic for malaria, microscopy results may push clinicians to think about non-malaria diagnoses in febrile patients with a positive RDT but very low parasitemia. In addition, light microscopy is crucial in epidemiologic surveys and hospitals where accurate speciation and quantification of samples is necessary. Handheld microscopy and mobile phone microscopy have recently been evaluated to overcome some of the logistic issues with laboratory-based diagnostics in resource-constrained settings.<sup>8–13</sup> The aim of these compact diagnostic tools is to provide

portable quality laboratory testing in rural, remote, or underserved settings rather than transferring ill individuals (or specimens) to distant reference laboratories. The goal of this study was to evaluate the “real-world” diagnostic operating characteristics of a handheld light microscope with mobile phone attachment integrated into a community-based screening program for malaria in rural Côte d'Ivoire.

### METHODS

The current study was embedded into an ongoing community-based cross-sectional epidemiologic study in the town of Grand Moutcho, Côte d'Ivoire (longitude 5.961, latitude –4.181), between April and June 2014. Ethical approval was granted by the Ministry of Health and Public Hygiene of Côte d'Ivoire (reference no. 32/MSLS/CNER-dkn). We obtained written informed consent from those older than 18 years and by the parents or legal guardians of children, who assented orally to participation. Those older than 6 years were invited to provide finger prick blood samples.

Before initiating the study, four Ivorian laboratory technicians received a half-day training with the Newton Nm1 microscope (Newton Microscopes; Cambridge, United Kingdom). This is a commercially available, handheld monocular light microscope with modular lenses (including a 100× lens) that weighs 480 g (Figure 1; www.newtonmicroscopes.com, accessed March 30, 2016). We attached an iPhone 5s (Apple, Cupertino, CA) to the microscope with the adaptor included with the Newton microscope. The camera function of the mobile phone is active while slides are maneuvered on the microscope stage, and images of the slide are visualized mobile phone screen in real time. The training involved a didactic session outlining how the handheld microscope functioned with and without a mobile phone attachment. This was followed by an interactive, hands-on teaching session where laboratory technicians had the opportunity to practice on at least 20 malaria test slides. The process was repeated until the disagreement between the expert microscopist and the technician was less than 10% on test slides. All training was under the

\*Address correspondence to Isaac I. Bogoch, Divisions of General Internal Medicine and Infectious Diseases, Toronto General Hospital, 14EN-209, 200 Elizabeth Street, Toronto, ON, Canada M5G 2C4. E-mail: isaac.bogoch@uhn.ca

†These authors contributed equally to this work.



FIGURE 1. The Newton Nm1 microscope in use, mounted on a tripod, at a rural clinic located in Grand Moutcho, Côte d'Ivoire.

direct supervision of an expert microscopist familiar with the device.

A single Giemsa-stained thick blood film was prepared from 223 individuals as per standardized protocols.<sup>14,15</sup> Laboratory technicians initially read each slide using an Olympus CX21 microscope (Olympus, Volketswil, Switzerland) under 1,000 $\times$  magnification with oil. All slides were evaluated on the same day of collection. Microscopists quantified malaria parasitemia per 200 white blood cells (WBCs), and in cases where fewer than 10 protozoa were visualized, parasitemia was quantified to 500 WBCs. Data were directly entered into an Excel database (Microsoft, Redmond, WA), and 10% of all slides were reexamined by a senior expert microscopist blinded to prior results using the Olympus CX21 microscope for quality control and validation. In cases of disagreement with the initial reading, the results were discussed with the individual technician, and the slides were read an additional time by both the laboratory technician and senior microscopist until an agreement was reached. All slides were coded and subsequently evaluated by laboratory technicians blinded to prior results using the Newton Nm1 microscope (1,000 $\times$  magnification with oil) with mobile phone attachment, as per the identical procedure listed above. We calculated the sensitivity, specificity, and positive and negative predictive values of the Newton Nm1 microscope with mobile phone attachment. In addition, linear association of malaria parasitemia calculated with the Newton Nm1 and the conventional microscope was assessed by Pearson's correlation coefficient. Quantitative agreement in parasitemia estimates obtained with the two diagnostic tools was further assessed by Bland–Altman plotting.

All analyses were done using R (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

We obtained 223 thick blood films from 223 individuals. The average age was 10 years (range: 5–19), with 108 (48.4%) women. Of 223 samples, 162 (72.6%) were positive for *P. falciparum* infection by conventional microscopy. Of the positive samples, 65 (40.1%) were of low parasitemia requiring counting to 500 WBC, and the overall mean parasite count was 1,392/ $\mu$ L of blood. Sensitivity and specificity of the Newton Nm1 microscope with mobile phone attachment were 80.2% (95% confidence interval [CI]: 73.1–85.9%) and 100% (95% CI: 92.6–100.0%), respectively, with a positive and negative predictive value of 100.0% (95% CI: 96.4–100.0%) and 65.6% (95% CI: 54.9–74.9%), respectively. Pearson's correlation comparing conventional microscopy with the Newton Nm1 microscope was 0.997. Despite strong linear correlation of estimates between the two devices, Bland–Altman plotting revealed that Newton Nm1 microscopy underestimated parasitemia, particularly at low parasitemia levels (Figure 2). A scatter plot comparing conventional light microscopy with the Newton Nm1 handheld microscope for *P. falciparum* diagnosis is shown in Figure 3.

## DISCUSSION

Malaria is a common and deadly protozoa infection in sub-Saharan Africa, and accurate diagnoses can enable swift treatment.<sup>16</sup> Here, we demonstrate that a handheld light microscope with a mobile phone attachment can be integrated into a routine use for a community-based malaria screening program to provide accurate diagnoses.

Handheld and mobile phone microscopy may be useful diagnostic tools in resource-constrained settings where laboratory infrastructure and equipment are limited. The aim of these portable devices is to bring quality laboratory diagnostics to underserved regions and avoid moving people long distances to the nearest laboratory. Several iterations of handheld microscopy have been validated in the past but unfortunately there has been little resulting momentum or scale-up.<sup>17,18</sup> Newer models of handheld and mobile-phone-based microscopes have recently been designed and evaluated for several infections with global health significance, including malaria, schistosomiasis, soil-transmitted helminthiasis, giardiasis, and *Loa loa*.<sup>8–10,12,13,19,20</sup> To date, most of these studies have been conducted in controlled laboratory settings, and the few field studies conducted used expert microscopists to operate novel diagnostic tools. Digitization of images (in this case, with a mobile phone camera) may be useful for several reasons, including integrating machine learning software for automated identification and quantification of pathogens, or for automated diseases mapping with geographic information systems. A major barrier to scale-up and implement these portable and handheld diagnostic devices is their validation by front-line health-care providers, who will ultimately use these tools in real-world settings.

We demonstrate that the Newton Nm1 handheld microscope with a mobile phone attachment has excellent operating characteristics as used by Ivorian laboratory technicians with a half-day training. The sensitivity of this device was

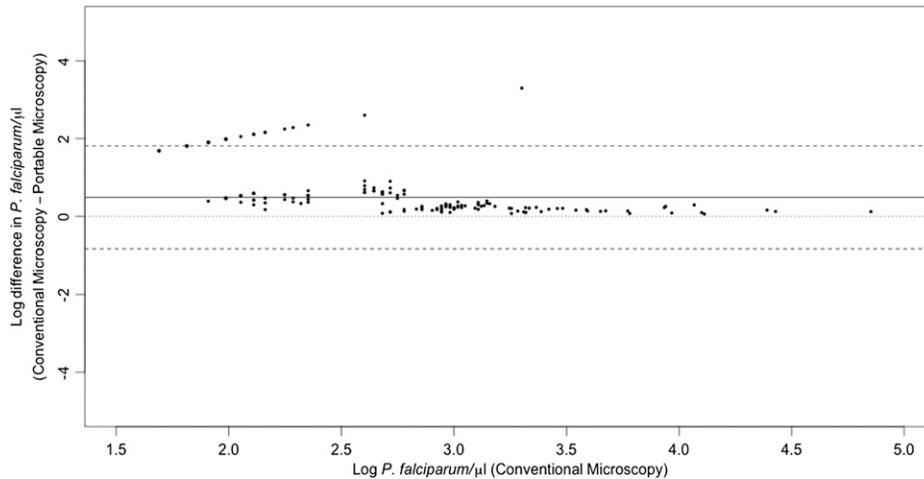


FIGURE 2. Bland–Altman plot comparing conventional light microscopy with the Newton Nm1 handheld microscope for the diagnosis of *Plasmodium falciparum* in a community-based cross-sectional screening program.

consistently over 80% by all microscopists when compared with conventional light microscopy, and the infections that were missed were low parasitemia infections. This study was conducted in a community-based rather than hospital-based setting where a greater proportion of individuals with high parasitemia would be expected. One other study evaluated the utility of this device; Ugandan laboratory technicians tested the Newton Nm1 on 50 preselected thick film test slides that had 30 positive and 20 negative samples and a mean parasite count of 1,464/ $\mu$ L of blood.<sup>12</sup> The laboratory technicians demonstrated an average sensitivity and specificity of 93.5% and 100%, respectively, and positive and negative predictive values of 100% and 90.5%, respectively. By comparison, our study had a slightly lower sensitivity and negative predictive value. Potential reasons include the added complexity of a mobile phone attachment to the microscope and a greater prevalence of lower intensity infections in the current study and that our study was conducted directly in the field, embedded in a cross-sectional survey, rather than under ideal

laboratory conditions using slides collected during prior field studies. Challenges we encountered in the field during the course of this study included frequent power outages and a laboratory environment that was exposed to environmental elements such as wind and the occasional ant infestation. While power outages may be disruptive to personnel, the Newton Nm1 microscope utilizes three AAA batteries with over 200 hours of power, and we did not have to change batteries during the course of the study. We also used tripods to support the microscopes and prevent microscopist fatigue. A concern with the lower sensitivity and negative predictive values is that there are false-negative readings in the context of a potentially deadly disease. While the device in its current state may not be ready for use in settings where high sensitivity is essential (e.g., clinical settings), the device may be helpful in field surveys to estimate the prevalence of disease in communities, with adjustments to balance for sensitivity limitations, to enable rapid prevalence mapping.

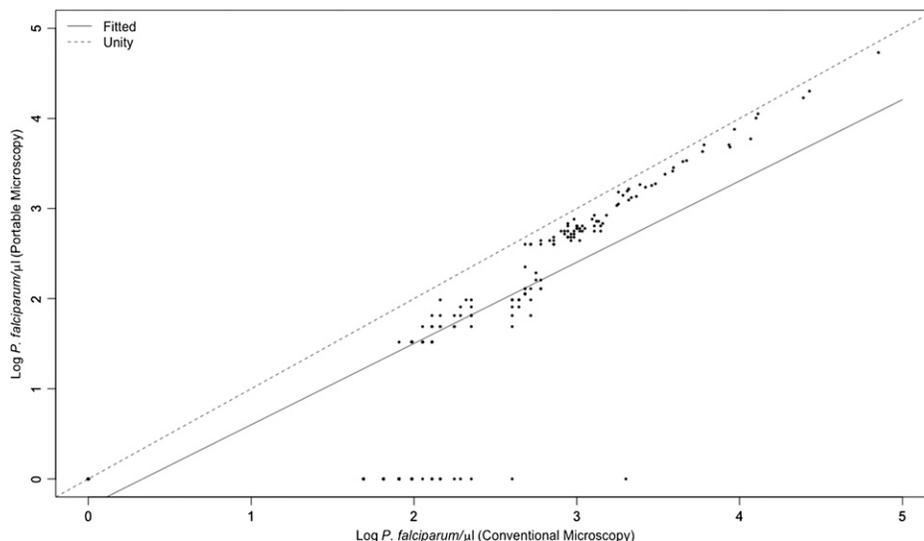


FIGURE 3. Scatter plot comparing conventional light microscopy with the Newton Nm1 handheld microscope for the diagnosis of *Plasmodium falciparum* in a community-based cross-sectional screening program.

Our study is limited in that we evaluated this handheld microscope in a community-based setting only. Future studies should assess the device in clinical settings, as used by front-line health-care personnel. In addition, future studies should focus on field applications of mobile-phone attachments harnessing computer learning technology for the automated identification and quantification of pathogens.<sup>19,20</sup> Finally, our study was conducted in a region of very high malaria prevalence and the majority of our population had low-grade parasitemia. Future studies should evaluate the operating characteristics of the Newton Nm1 microscope in regions with lower malaria prevalence.

Handheld light microscopy with a mobile phone attachment can be integrated into routine practice of community-based screening programs to diagnose malaria infections. This valuable diagnostic tool may be helpful for malaria control efforts in resource-constrained environments.

Received April 26, 2016. Accepted for publication June 16, 2016.

Published online August 15, 2016.

Financial support: Jean T. Coulibaly is supported by the Programme d'Appui Stratégique à la Recherche Scientifique (PASRES), Côte d'Ivoire (reference no. 113). Isaac I. Bogoch is supported by Grand Challenges Canada, Stars in Global Health, 0631-01-10 (www.grandchallenges.ca) and a grant from the MSH UHN AMO Innovation Fund.

Disclaimer: The funders played no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' addresses: Jean T. Coulibaly, Mamadou Ouattara, and Eliézer K. N'Goran, Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire, E-mails: couljeanvae@yahoo.fr, mamadou\_ouatt@yahoo.fr, and eliezerngoran@yahoo.fr. Jennifer Keiser, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland, E-mail: jennifer.keiser@unibas.ch. Bassirou Bonfoh, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire, E-mail: bassirou.bonfoh@csrs.ci. Jason R. Andrews, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, E-mail: jandr@stanford.edu. Isaac I. Bogoch, Division of General Internal Medicine, Toronto General Hospital, Toronto, Canada, and Division of Infectious Diseases, Toronto General Hospital, Toronto, Canada, E-mail: isaac.bogoch@uhn.ca.

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