

## Short Report: Quantitative Evaluation of a Handheld Light Microscope for Field Diagnosis of Soil-Transmitted Helminth Infection

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**Abstract.** We evaluated the Newton Nm1, a commercially available handheld light microscope and compared it with conventional light microscopy for the diagnosis of soil-transmitted helminth infections. A total of 91 Kato-Katz thick smears were examined by experienced microscopists and helminth eggs were counted and expressed as eggs per gram of stool (EPG). Mean egg counts were significantly higher with the conventional light microscope (5,190 EPG versus 2,386 EPG for *Ascaris lumbricoides*; 826 versus 456 for *Trichuris trichiura*; both  $P < 0.05$ ). Using regression coefficients and accounting for intensity of infection, we found that the agreement between the two devices was excellent for both species ( $\kappa = 0.90$ , 95% confidence interval = 0.82–0.99 for *A. lumbricoides* and  $\kappa = 0.96$ , 95% CI = 0.91–1.00 for *T. trichiura*). The Newton Nm1 microscope may be a useful tool for the detection and quantification of soil-transmitted helminth infection in clinical, epidemiologic, and public health settings.

### INTRODUCTION

Chronic infection with soil-transmitted helminths (STHs; *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm) pose considerable public health burden.<sup>1</sup> More than one billion persons are infected, and children and impoverished and marginalized populations are disproportionately affected.<sup>1,2</sup> Sustained, high-intensity infection may lead to malnutrition and anemia, which can stunt growth and cognitive development.<sup>3,4</sup> Accurate diagnosis of STH infection is important for clinical and epidemiologic purposes, and for monitoring control programs.<sup>5</sup>

Currently, stool light microscopy using the Kato-Katz thick smear preparation<sup>6</sup> is the most widely used, but it is an imperfect diagnostic test in epidemiologic surveys. Multiple Kato-Katz thick smears are required to increase diagnostic sensitivity.<sup>7,8</sup> Light microscopy is typically performed in laboratory settings distant from where most STH-infected persons reside. Handheld microscopic devices may help overcome barriers to care because devices can be transported to affected regions easily, do not require a constant supply of electricity, and may serve as a point-of-care test.<sup>9,10</sup> We quantitatively compared fecal egg counts obtained by using a handheld light microscopic device (i.e., Newton Nm1) with those obtained by using conventional light microscopy for the diagnosis of *A. lumbricoides* and *T. trichiura* infections on Pemba Island, United Republic of Tanzania.

### METHODS

This study was part of a clinical trial evaluating anthelmintic medications against STH infections in school-age children on Pemba Island, Tanzania. Ethical approval was

granted by the ethics committee of Basel (EKBB; reference no. 123/13), and the Ministry of Health and Social Welfare of Zanzibar (ZAMREC; reference no. 0001/June/13). The trial was conducted during September 2013–January 2014 at two schools and included children 6–14 years of age. Parents provided written informed consent and children assented orally for voluntary participation. The trial is registered at Current Controlled Trials (identifier: ISRCTN80245406). All infected children were treated with albendazole (400 mg) free of charge.

Two consecutive morning stool samples were collected from children. Samples were transferred to the Ivo de Carneri Public Health Laboratory for processing and analysis on the day of collection. Two Kato-Katz thick smears (using 41.7 mg of standard templates) were prepared from each stool specimen, and each was quantitatively examined by experienced laboratory technicians under a light microscope for *A. lumbricoides*, *T. trichiura*, and hookworm eggs. Kato-Katz thick smears were examined within 60 minutes of preparation to account for rapidly clearing hookworm eggs.<sup>11</sup> A total of 10% of Kato-Katz thick smears were randomly chosen and re-examined by a senior laboratory technician to ensure high quality of helminth egg identification and quantification.

Ninety-one slides were randomly selected after diagnosis by using conventional microscopy and re-examined with the Newton Nm1-600 XY Portable Field Microscope (Newton Microscopes, Cambridge, United Kingdom) by an experienced microscopist. This handheld, commercially available device has modular objective lenses and can manipulate sample slides in the x and y axes. The microscopist was blinded to results from conventional light microscopy. Only *A. lumbricoides* and *T. trichiura* were evaluated given the time delay of several hours and the risk of rapidly clearing hookworm eggs.<sup>11</sup>

All fecal egg counts from conventional microscopy and the Newton Nm1 were double-entered into an Excel spreadsheet (Microsoft, Redmond, WA). Data were analyzed with Stata version 10.1 (StataCorp LP, College Station, TX). All egg counts were multiplied by a factor of 24 to obtain eggs per

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gram of stool (EPG). We compared egg counts by using a Wilcoxon sign rank test. We then assessed linear correlation in fecal egg counts between the conventional light microscope and Newton Nm1 by using Pearson's correlation coefficient, and estimated a regression coefficient through linear regression. We categorized infections according to intensity (light, moderate, and heavy infection) according to World Health Organization guidelines.<sup>12</sup> Moderate-intensity and heavy-intensity infections were grouped together because there were only two heavy infections, as determined by conventional light microscopy for *A. lumbricoides* and *T. trichiura*. We calculated a Kappa statistic comparing classification of infection intensity for the two types of microscope. In addition, we generated adjusted fecal egg count estimates from the Newton Nm1 by multiplying the observed values by the regression coefficient, and re-calculated the Kappa statistic by using re-classified infection intensity. We report unadjusted and adjusted Kappa values.

## RESULTS

Conventional light microscopy showed that 35 (38.5%) of 91 Kato-Katz thick smears contained *A. lumbricoides* and 70 (76.9%) of 91 thick smears contained *T. trichiura* eggs. Using the Newton Nm1 handheld microscope, we found that 36 (39.6%) and 64 (70.3%) were positive for *A. lumbricoides* and *T. trichiura*, respectively (Table 1).

The mean fecal egg count for both infections was significantly higher with the conventional light microscope (5179 EPG versus 2,385 EPG for *A. lumbricoides* and 826 versus 456 for *T. trichiura*;  $P = 0.017$  and  $P = 0.004$ , respectively). However, Pearson's  $r$  suggested an excellent correlation between the devices for *A. lumbricoides* ( $r = 0.90$ ) and *T. trichiura* ( $r = 0.94$ ). Regression coefficients for the Newton Nm1 microscope estimates were 2.03 ( $P < 0.001$ ) for *A. lumbricoides*, and 1.56 ( $P < 0.001$ ) for *T. trichiura*. Agreement between Newton Nm1 handheld microscopy and conventional light microscopy for intensity of infection was good for *A. lumbricoides* ( $\kappa = 0.82$ , 95% confidence interval [CI] = 0.71–0.93) but only moderate for *T. trichiura* ( $\kappa = 0.47$ , 95% CI = 0.30–0.63). After adjusting fecal egg counts by using

regression coefficients and re-classifying intensity of infection, we found that the correlation between the two devices was excellent for both helminth species ( $\kappa = 0.90$ , 95% CI = 0.82–0.99 for *A. lumbricoides* and  $\kappa = 0.96$ , 95% CI = 0.91–1.00 for *T. trichiura*).

## DISCUSSION

Soil-transmitted helminthiasis remains a major global health concern,<sup>1,2</sup> and most affected persons reside in regions without access to conventional diagnostic facilities. Portable microscopic devices may be a useful modality to provide diagnostic support in remote, rural, or underserved locations for clinical, public health, and epidemiologic applications.<sup>13</sup> We demonstrated an excellent correlation in the quantitative diagnosis of STH infections between the Newton Nm1 handheld microscope and conventional light microscopy.

Our previous data demonstrated excellent diagnostic sensitivity and specificity of the Newton Nm1 handheld microscope for schistosomiasis (*Schistosoma mansoni* in Kato-Katz thick smears and *S. haematobium* in filtered urine) and STH infections.<sup>14</sup> However, egg burdens have not yet been quantified by using this novel device. Accurate quantification of helminth infection intensity is vital if such a device is to be used in clinical, public health, and epidemiologic settings because infection intensity, which is proxied by egg counts, is correlated with morbidity.<sup>15</sup> Moreover, precise quantification of egg counts is of pivotal importance to estimate egg reduction rates, clinical relevance of symptoms, and the accuracy of mapping infection prevalence and intensity in populations.<sup>1,16</sup> We used a tripod for stability and to ensure that the microscope was at a comfortable level for the microscopist while examining many slides.

We found that the mean fecal eggs counts for *A. lumbricoides* and *T. trichiura* were considerably lower when using the Newton Nm1 microscope compared with using conventional light microscopy. However, the measurements were reliably lower and showed excellent linear correlation between estimates. We therefore were able to apply a linear multiplier to fecal egg counts obtained with the Newton Nm1 microscope to generate egg counts that were comparable to those obtained

TABLE 1

Diagnostic characteristics of the Newton Nm1 handheld microscope and conventional light microscopy for different infection intensities of *Ascaris lumbricoides* and *Trichuris trichiura*

<i>Ascaris lumbricoides</i>	Newton Nm1 handheld microscope				Total
		Negative	Light	Moderate/heavy	
Conventional light microscope	Negative	54	2	0	56
	Light†	1	17	0	18
	Moderate/heavy‡	0	6	11	17
	Total	55	25	11	91
Unadjusted Kappa = 0.82, 95% CI* = 0.71–0.93, adjusted Kappa = 0.90, 95% CI = 0.82–0.99					
<i>Trichuris trichiura</i>	Newton Nm1 handheld microscope				Total
		Negative	Light	Moderate/heavy	
Conventional light microscope	Negative	17	4	0	21
	Light§	10	39	0	49
	Moderate/heavy¶	0	14	7	21
	Total	27	57	7	91
Unadjusted Kappa = 0.47, 95% CI = 0.30–0.63, adjusted Kappa = 0.96, 95% CI = 0.91–1.00					

\*CI = confidence interval.

†1–4,999 eggs/gram of stool.

‡≥ 5,000 eggs/gram of stool.

§1–999 eggs/gram of stool.

¶≥ 1,000 eggs/gram of stool.

by from conventional microscopy. After this adjustment, correlation in intensity of infection was excellent (e.g., adjusted Kappa values  $\geq 0.90$ ), suggesting a good fit for our model.

Several reasons account for the lower direct counts of egg burden when using the Newton microscope. These reasons include the degree of x and y axes manipulation in the device, and how Kato-Katz thick smears are prepared. The Newton Nm1 handheld microscope has the ability to manipulate slides in the x and y axes but to a lesser degree compared with conventional light microscopes. When a Kato-Katz thick smear is being prepared, 41.7 mg of stool is placed on a microscope slide with a cut out template and compressed.<sup>17</sup> If the stool specimen is compressed over a wide surface area on the slide, the Newton Nm1 handheld microscope may not be able to detect eggs that are at the very periphery of the sample because of limitations in how far the stage can move in the x and y axes. This limitation may result in counting fewer eggs on the slide, and because of the high multiplication factor (i.e., 24), result in lower EPG estimates. This was evident for *A. lumbricoides* and *T. trichiura* in our study. In addition, if there are low egg intensities and eggs are peripherally located on the slide, the Newton Nm1 handheld microscope may count a sample as negative for infection. This raises the important issue that multiple samples should be evaluated to maximize sensitivity and specificity of the device similar to the Kato-Katz technique.<sup>7</sup> Furthermore, if slides were prepared with the stool specimen centered such that the entire sample could be evaluated within the range of x and y axes of the stage, it is likely that no statistical calibration would be required. We would expect similar positive results for hookworm and *S. mansoni* infection given the excellent diagnostic properties of the Newton Nm1 handheld microscope and utility of the correction factor for *A. lumbricoides* and *T. trichiura*, but this expectation still requires validation.

Future studies should evaluate the utility of this device in real world settings by healthcare professionals in clinical environments, and public health teams in epidemiologic settings. In addition, mobile phones are being converted into portable microscopes.<sup>10</sup> Newer technologies use innovative image capturing techniques, such as lens-free devices, which provide quality diagnostics in laboratory settings, but will require rigorous validation in clinical and field settings.<sup>18–21</sup> These devices have the benefit of digitizing images upfront, which may enable real-time data sharing and could facilitate more rapid diagnoses,<sup>22</sup> medical education, or real-time infection mapping of regions.

In conclusion, the Newton Nm1 handheld microscope has the ability to accurately quantify infection with *A. lumbricoides* and *T. trichiura* such that it could be considered a helpful tool in clinical, public health, and epidemiologic settings. Further validation is required for other helminth infections, intestinal protozoa, and the malarial parasite *Plasmodium* as multiparasitism is the norm rather than the exception in remote rural areas of the tropics.

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