

# Foldscope: Diagnostic Accuracy and Feasibility of its Use in National Malaria Control Program

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## Abstract

**Background:** Malaria has been an important public health all over the globe. Although conventional light microscopy is the gold standard of diagnosis, light microscopes are heavy, fragile, costly, and electricity dependent. Rapid diagnostic tests (RDTs) have become more popular but perform badly in temperate climate. This is because the RDT kits require maintenance of cold chain for its optimal use. In this regard, there is a recent interest in handheld malaria microscopy at the point of care in the field setting. Foldscopes are cheap, handy, nonfragile, and use mobile camera for illumination. The purpose of the study was to find whether foldscope can be used in the national vector borne disease control program (NVBDCP) in India. **Methods:** Ten laboratory technicians were trained in identifying malaria parasites using foldscope and their mobiles. Later, they were provided with unassembled foldscope to document their test results for the preidentified malaria slides. The blood smears were stained as per the protocol of NVBDCP. The report of the index test (foldscope microscopy) was compared with the reference test (conventional microscopy). **Results:** The sensitivity and specificity of the index test was found to be 13.3% (6.257–26.18), specificity of 97.78% (88.43–99.61), positive predictive value 85.71% (48.69–97.43), and negative predictive value 53.01% (42.38–63.38). The device failure rate and test failure rate were 20% and 11.7%. The kappa agreement between the index and reference microscopy was only 11% and the McNemar  $P < 0.01$ . **Conclusion:** The  $\times 400$  foldscope at its present magnification and illumination cannot be utilized in the field under NVBDCP.

**Keywords:** Field study in malaria, foldscope, handheld microscope, malaria slide examination, malaria, national vector borne disease control program

## INTRODUCTION

Malaria is a public health problem in several parts of the globe, especially in Afro-Asian countries. About 95% of the Indian population reside in malaria endemic areas.<sup>[1]</sup> About 20% of the population of India reside in hilly and tribal areas. However, out of all malaria cases, about 80% of total malaria cases are reported from these hilly, tribal, and difficult to access areas of the country.<sup>[1]</sup> Malaria parasite detection by conventional binocular bright field microscope using thick and thin smears has been the gold standard.<sup>[2]</sup> However, the high cost of microscopy, repeated power failure in rural areas, fragility of microscope, and high cost of maintenance have opened the door for rapid diagnostic tests (RDT). Although RDTs have replaced the conventional binocular bright field microscopy

over years in the national malaria control programs, high ambient temperature and poor maintenance of cold chain in storing the RDTs make them vulnerable to false result. In addition to that for the epidemiological survey, malaria microscopy is the key.

The conventional microscopes are heavy, fragile, costly, and electricity dependent. In the last decade or so, there has been a growing interest on handheld, handy, and cheap microscopes.<sup>[3-7]</sup> Some of the devices even use mobile phone cameras to illuminate the object.<sup>[3,5,8]</sup> Handheld microscopes with earlier mentioned advantages have been an exciting

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entry in the field of malaria microscopy. Foldscopes are origami-based paper microscopes developed by Dr. Manu Prakash and team at Stanford University.<sup>[3]</sup> Foldscopes are cheap about 500 Indian national rupees, light about 10 g, and easy to carry in a laboratory coat which uses mobile flashlight for illumination.<sup>[3]</sup> It is based on the principles of optical design and origami. The sample is mounted on a slide and viewed while panning and focusing with the operator's thumb with a magnifying scalability of 140–2180 times without the requirement of immersion of oil as in Figure 1.<sup>[3]</sup>

Most of the handheld microscopes including foldscope have been studied in controlled laboratory settings and by experienced hands. There are absolutely scanty field-based studies for malaria with handheld microscopes. In this context, the present study was undertaken to evaluate the diagnostic accuracy of origami-based paper foldscope (index test) in comparison to the conventional binocular bright field microscope (reference test) and its feasibility to use for national malaria control program in India.

## METHODS

### Design

This was a cross-sectional diagnostic accuracy and feasibility study.

### Setting

The study was conducted at the Microbiology Laboratory of the All India Institute of Medical Sciences, Bhopal, and malaria microscopy centers of Bhopal district recognized by the Government of MP under the national vector borne disease control program (NVBDCP) of the state.

### Sampling and sample size

The slide positivity rate was approximately 0.5% for malaria parasite.<sup>[1]</sup> With an expected malaria slide positivity of approximately 0.5, sensitivity of 90% and specificity of 90%, and precision of 90% with a confidence interval of 95%, the required sample size was calculated to be 102. The same number of samples were collected for the study purpose. A consecutive sampling technique was used.

### Study procedure

The technicians identified by NVBDCP of the state were provided a full 1-day hands-on training to use foldscope by arranging a workshop. They were trained to the level of agreement for 90% to detect positive and negative results for malaria parasites using both the reference microscope (conventional light microscope) and index microscope ( $\times 400$  foldscope). Later, they were provided unassembled foldscope and advised to comment on the slides preidentified by NVBDCP. Both the Ethylene Diamine Tetra Acetic acid (EDTA) mixed venous blood smear or peripheral blood smear slides were accepted for the study. The blood smears were stained by Jaswant Singh–Bhattacharji (JSB) stain as per the protocol of NVBDCP. They were asked to document observations as positive/negative/device failure and test

failure. Device failure was defined by the inability of the field technician to get any image of the slide and test failure was defined by the inability of the field technician to report positive or negative for malaria parasite.

### Analysis

The diagnostic screening test was evaluated by constructing a  $2 \times 2$  table after excluding the test failure cases. The table was finally analyzed by OpenEpi Version 3. Kappa agreement and McNemar's two-sided *P* value were calculated by IBM SPSS Statistics Version 24. The confidence interval was set at 95% and level of significance  $P < 0.05$ .

## RESULTS

Out of the total 102 MP slides, 49 cases were positive and 53 cases were negative for MP in reference microscopy. Out of the total 49 positive cases in reference microscopy, only 6 slides were positive by index microscopy test (true positive), 39 cases were reported to be negative (false negative), and 4 cases could not be commented either positive or negative. Out of the total 53 negative cases in reference microscopy, 44 slides were negative by index microscopy test (true negative), 1 case was reported to be positive (false positive), and 8 cases could not be commented either positive or negative. The values of sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratios are provided in Table 1.

The device failure rate was 20% (2/10), meaning that 2 of the technicians out of the total 10 technicians required help later for assembling the foldscope. Test failure cases were 12 in number meaning that the index microscopy could not comment on either positive or negative test. The test failure rate was 11.7% (12/102). The various diagnostic test characteristics are provided in Table 1. The Kappa measure of agreement between the reference and index microscopy tests was 11.1% (0%–22.3%). The McNemar 2-sided *P* was significant ( $<0.001$ ).

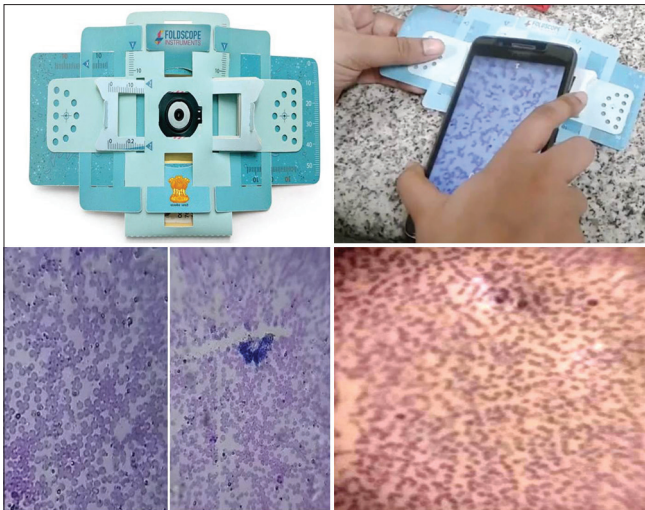
## DISCUSSION

Handheld microscopy at the point of care is the need of

**Table 1: Characteristics of the index test**

Parameter	Estimate (%)	95% CIs (lower-upper)
Sensitivity	13.33	6.257-26.18
Specificity	97.78	88.43-99.61
Positive predictive value	85.71	48.69-97.43
Negative predictive value	53.01	42.38-63.38
Diagnostic accuracy	55.56	45.27-65.38
Likelihood ratio of a positive test	6	0.1011-356.1
Likelihood ratio of a negative test	0.8864	0.8421-0.933
Kappa agreement (%)	11	0-23
Level of significance McNemar <i>P</i>		<0.001

CI: Confidence interval



**Figure 1:** Foldscope figure

the hour. Various researches have tried many devices using different principles of optics to study infectious and other environmental agents.<sup>[3-21]</sup> However, there is only handful of patient-related studies about infectious agents. Majority of them deal with human parasites.<sup>[3,4-7]</sup> Interestingly, all of these devices were smartphone compliant. We could identify only one study related to malaria parasite in field conditions by Coulibaly *et al.*<sup>[8]</sup> In our study with foldscope, the device failure rate was 20%. As this is a new technique, never used previously by the technicians, the device failure rate was pure because of assembly techniques which were tackled easily over the phone by video call. The device failure rate of 20% seems acceptable which could have been reduced further on the distribution of prerecorded video messages on the assembly technique. The test failure rate in our study was 11.7%. It means that in 12 out of 100 cases, the technician was not sure whether the test is positive or negative. Earlier studies using handheld devices for malaria or other parasitic infections did not document the device failure rate and test failure rates and hence could not be compared.

As described in Table 1, the sensitivity and specificity of our study were 13.3% and 97.7%, respectively. Coulibaly *et al.* (2016) described the sensitivity and specificity of the handheld light microscope as 80.2% and 100% respectively. The difference seems to be because of different types of the handheld microscope (Newton Nm1 microscope) and better quality of mobile used by Coulibaly *et al.* (Samsung in our study vs. iPhone). It is also to be noted that Coulibaly *et al.* engaged only 4 technicians for double the sample size than that of ours (10 technicians in our study). With the sensitivity of 13.3%, the foldscope at this present form does not seem up to mark to be used as a screening technique to replace gold standard conventional microscopy. Although the specificity looks impressive at 97.7%, with the negative predictive value of over 50%, it also lacks the credibility of a confirmatory test. It means it will be difficult to find out true negative cases. The positive predictive value of 85.7% along with the extremely

low sensitivity of 13.3% means a lot of positive cases will be missed while screening which has a huge implication in the field settings. As per Coulibaly *et al.*, the positive and negative predictive values were 100% and 65.6%, respectively, which definitely seem to be better than values of this work. The possible explanation of better result by Coulibaly *et al.* has already been explained above. The positive and negative likelihood ratio of our study was 6 and <1, respectively. As the negative likelihood ratio is <1, the treating physician can never be sure withholding treatment in case of a negative test. The likelihood ratios have not been documented by Coulibaly *et al.* and hence could not be compared.

The agreement between the gold standard reference microscopy and index microscopy (foldscope) is only 11% in the current study. Coulibaly *et al.* also documented disagreement by the Bland–Altman plotting in spite of a linear correlation ship with Pearson's 0.997. In the present study, McNemar test *P* was <0.001 which is highly significant. It seems the ×400 foldscope at the present stage is not good enough for field settings with an extremely low sensitivity. However, as other forms of foldscope can magnify to the extent of 2000 times, it seems extremely interesting to test other foldscopes in field setting.

## CONCLUSION

The foldscope is feasible to be used in a field setting under NVBDCP. However, the diagnostic accuracy is low with sensitivity of 13%, specificity of 97%, positive predictive value of 85%, and negative predictive value of 53%. As the agreement between the gold standard binocular bright field reference microscopy and index bright field foldscope is extremely low (11%) with significant McNemar test *P*, the foldscope in its present magnification and illumination cannot be utilized under NVBDCP for malaria microscopy in India. Similar conclusion may also be derived for malaria control programs across the globe.

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## Conflicts of interest

There are no conflicts of interest.

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